LIBRARY
FISH & WILDLIFE SERVICE
PACIFIC OCEANIC FISHERY
INVESTIGATIONS

PERIODICAL
Calif. Univ.
S.I.O.
Contributions
1943

DATE [redacted]
BORROWER'S NAME
[redacted]

PERIODICAL

Calif. Univ. S.I.O.
Contributions

1943

LIBRARY
BUR. OF COMM. FISH.
HON. BIOL. LAB.

LIBRARY
FISH & WILDLIFE SERVICE
PACIFIC OCEANIC FISHERY
INVESTIGATIONS

SCRIPPS INSTITUTION OF OCEANOGRAPHY

UNIVERSITY OF CALIFORNIA

CONTRIBUTIONS

1943

1717

This volume contains contributions from the Scripps Institution of Oceanography which appeared during 1943 in publications other than *Bulletins of the Scripps Institution of Oceanography* and *Records of Observations of the Scripps Institution of Oceanography.*

LA JOLLA, CALIFORNIA
March, 1944

TABLE OF CONTENTS

Contribution No.

ALLEN, W. E.
Microscopic creatures afloat in water 188
"Red water" in La Jolla Bay in 1942 213

ANDERSON, L. J. with J. C. HINDMAN
A versatile continuous reading thermionic voltmeter 189

COE, WESLEY R.
Development of the primary gonads and differentiation of sexuality in *Teredo navalis* and other pelecypod mollusks 193
Sexual differentiation in mollusks. I. Pelecypods 197

FOX, DENIS L., and W. R. COE
Biology of the California sea-mussel (*Mytilus californianus*). II. Nutrition, metabolism, growth and calcium deposition 205

FOX, DENIS L., and D. M. UPDEGRAFF
Adenochrome, a glandular pigment in the branchial hearts of the octopus 184

GILBERT, J. Y., and W. E. ALLEN
The phytoplankton of the Gulf of California obtained by the *E. W. Scripps* in 1939 and 1940 183

GRAHAM, HERBERT W.
Chlorophyll-content of marine plankton 200
Gymnodinium catenatum, a new dinoflagellate from the Gulf of California 212

JACOBS, WOODROW C.
Sources of atmospheric heat and moisture over the North Pacific and North Atlantic Oceans 201

JOHNSON, MARTIN W.
Studies on the life history of the marine annelid *Nereis vexillosa* 191

McEWEN, GEORGE F.
Statistical problems of standardizing the precipitation ratio and estimating its error . . 206

NOVELLI, G. DAVID
Bacterial oxidation of hydrocarbons in marine sediments 196

SARGENT, MARSTON C., and J. C. HINDMAN
The ratio of carbon dioxide consumption to oxygen evolution in sea water in the light . 198

SHEPARD, FRANCIS P.
Imaginary submarine canyons 214
Shoreline erosion at La Jolla, Calif. 186

SUMNER, F. B.
A further report upon the effects of the visual environment on the melanin content of fishes 195

SUMNER, F. B., and PETER DOUDOROFF
An improved method of assaying melanin in fishes 194

Contribution No.

SVERDRUP, H. U.
On the ratio between heat conduction from the sea surface and heat used for evaporation . . 202

SVERDRUP, H. U., and STAFF
Research within physical oceanography and submarine geology at the Scripps Institution of Oceanography during April 1942 to April 1943 207

TSCHUDY, ROBERT H., and M. C. SARGENT
Agar-bearing seaweeds at La Jolla, California 187

WORLEY, LEONARD G.
The relation between the Golgi apparatus and "droplets" in the cell stainable vitally with methylene blue 209
The structure and function of the Golgi system in the living cells of developing molluscs . 208

WORLEY, LEONARD G., and ELIZABETH K. WORLEY
Studies of the supravitally stained Golgi apparatus. I. Its cycle in the tectibranch mollusc, *Navanax inermis* (Cooper) 211

YOUNG, R. T., JR.
A note on some laboratory measurements of the transparency of sea water 203

ZOBELL, CLAUDE E.
The effect of solid surfaces upon bacterial activity 204
Influence of bacterial activity on source sediment 192

ZOBELL, CLAUDE E., and C. W. GRANT
Bacterial utilization of low concentrations of organic matter 199

ZOBELL, CLAUDE E., C. W. GRANT, and H. F. HAAS
Marine microorganisms which oxidize petroleum hydrocarbons 210

Contribution No. 183

THE PHYTOPLANKTON OF THE GULF OF CALIFORNIA OBTAINED BY THE "E. W. SCRIPPS" IN 1939 AND 1940*

By

J. Y. GILBERT AND W. E. ALLEN
Scripps Institution of Oceanography
University of California
La Jolla, California

The waters of the Gulf of California are fabulously rich in marine life; they fairly teem with multitudes of fish of both commercial and sport varieties. To maintain these large numbers there must be correspondingly huge crops of their ultimate food—the phytoplankton. And, indeed, it was the enormous quantity of these microscopic plants that gave the Gulf its early and descriptive name—the Vermilion Sea. In 1939 and again in 1940 the "E. W. Scripps," research vessel of the Scripps Institution of Oceanography of the University of California, spent several months in these waters and among the data collected on these cruises were quantitative samples of this phytoplankton. It is with these samples that the present report deals.

The phytoplankton of the Gulf, or rather the diatoms which in this region are its main constituent, also plays a considerable geologic role. Bottom samples taken on these two cruises revealed that diatomaceous sediments are being laid down over large areas in the Gulf, probably under conditions similar to those which gave rise to the Miocene diatomaceous shales of the California coast region (Sverdrup and Staff, 1940).

---

* Contribution from the Scripps Institution of Oceanography, New Series, No. 183.

Reprint from SEARS FOUND. JOURN. MAR. RES.

Prior to 1939 four series of phytoplankton samples had been collected in the Gulf of California. The first series was obtained in 1921 between April 7 and July 11 by the Expedition of the California Academy of Sciences (Allen, 1923); the second in November of 1935 by the Templeton Crocker Expedition (Allen, 1938); the third in 1936 between February 8 and March 20 by the Allan Hancock Expedition of 1936 (Allen, 1937); and the fourth by the Allan Hancock Expedition of 1937 (Cupp and Allen, 1938) between March 1 and April 4.

The 1939 cruise (Figure 30) of the "E. W. Scripps" was of a general oceanographical nature and between February 13 and March 19 the ship occupied 53 stations, which were spaced 15 to 20 miles apart along 9 lines across the Gulf; at 49 of these stations phytoplankton samples were obtained. For the first time the Gulf was systematically sampled and for the first time phytoplankton samples were collected from the surface down to 60 meters at intervals of 10 meters. All of the preceding series had consisted entirely of surface samples. The 1940 cruise (Figure 31), from October through December, was primarily concerned with the geology of the Gulf, but at twenty-two stations was phytoplankton collected in a somewhat limited area. At ten of these stations surface samples only were obtained.

The methods of collecting and enumerating the organisms were similar to those employed on the previous expeditions to the Gulf. Five liters of water, collected with the Allen closing bottle, were filtered through a net of No. 25 bolting silk and the concentrated sample preserved with a small quantity of formalin. The phytoplankton content of these concentrated samples was later determined by enumeration of the organisms in an aliquot portion in a Sedgwick-Rafter counting chamber.

A phytoplankton population is a crop in three dimensions and should be appraised from a number of samples varying in position in both horizontal vertical directions. Often when it is not possible, for one reason or another, to sample subsurface depths, the size of the population at the surface is used as an index of the total population in a water column and in this manner valuable information may be gained. Behind this latter procedure is the assumption, usually implicit, that the total population is proportional to the surface population. The use of this assumption is justified when the opportunity for more thorough sampling is lacking as was the case in the Gulf cruises prior to the 1939 cruise of the "E. W. Scripps." A difficulty arises, however, when these results, based on surface samples alone, are to be compared with the results of the 1939 and 1940 cruises when subsurface depths were sampled.

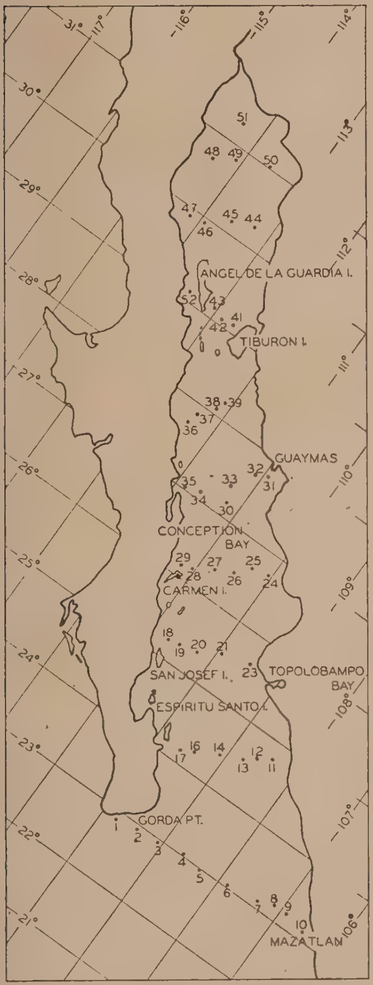

31°
117°
116°
115°
114°
113°
112°
111°
110°
109°
108°
107°
106°
30°
29°
28°
27°
26°
25°
24°
23°
22°
21°
ANGEL DE LA GUARDIA I.
TIBURON I.
GUAYMAS
CONCEPTION BAY
CARMEN I.
SAN JOSEF I.
ESPIRITU SANTO I.
TOPOLOBAMPO BAY
GORDA PT.
MAZATLAN
51
48
49
50
47
46
45
44
52
43
42
41
38
39
37
36
35
33
32
31
34
30
29
27
25
28
26
24
18
19
20
21
23
16
17
14
12
13
11
1
2
3
4
5
6
7
8
9
10

Figure 30. Location of stations at which phytoplankton samples were collected on the *E. W. Scripps* Cruise VII in 1939.

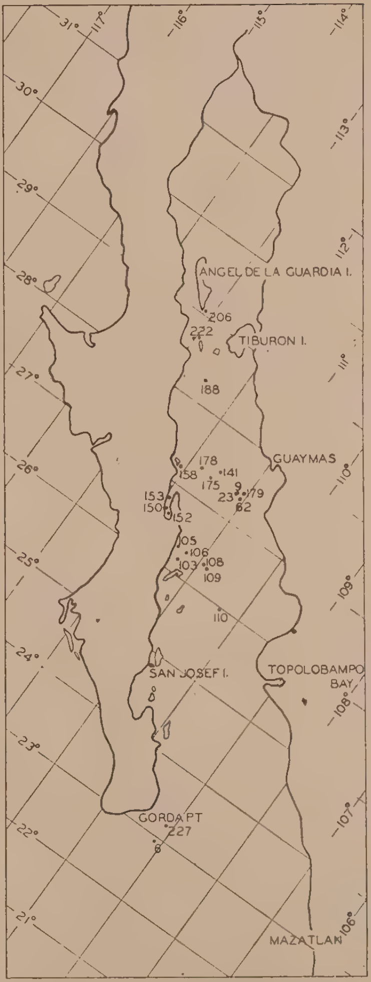

31°
117°
116°
115°
114°
113°
112°
111°
110°
109°
108°
107°
106°
30°
29°
28°
27°
26°
25°
24°
23°
22°
21°
ANGEL DE LA GUARDIA I.
TIBURON I.
GUAYMAS
SAN JOSEF I.
TOPOLOBAMPO BAY
GORDA PT
MAZATLAN
206
222
188
178
158
141
175
23
9
179
62
153
150
152
105
106
103
108
109
110
227
8

Figure 31. Location of stations at which phytoplankton samples were collected during the *E. W. Scripps* Cruise XVI in 1940.

A logical expression for the size of the total standing crop of phytoplankton is the number of plant cells under a certain area of the sea surface (cf. Bigelow et al., 1941) but this number obviously cannot be compared with the surface population of a previous cruise. A better statistic for this purpose is the mean number of cells per liter in the water column sampled. In practice the depth sampled is usually less than 100 meters, as by far the major portion of the population is found above this depth, and in the waters off Southern California it has been found that the number of plant cells below 60 meters is usually so slight that the time spent in sampling depths greater than this might be more profitably spent in increasing the number of water columns sampled. The mean number of cells per liter for this 60 meter column of water is generally of the same order of magnitude as the surface population and one will not err too greatly in comparing the two if their distinction is kept in mind. This mean number of cells per liter was calculated as a weighted mean from the formula:

$$M = \frac{\frac{1}{2}(N_0 + N_{60}) + N_{10} + N_{20} + N_{30} + N_{40} + N_{50}}{6}, \text{ where } N_z \text{ is}$$

the number of cells per liter at the depth indicated by the subscript z.

It is not to be expected that the data obtained on these two cruises, even when combined with those of the previous cruises, will yield a complete picture of the phytoplankton of the Gulf of California. It should hardly be necessary to point out that a large number of observations are required to achieve any valid understanding of the distribution and behavior of the plankton in any region. In the laboratory one variable can be isolated by keeping constant the more important of the remaining variables, and from a relatively few measurements the responses of the organism to changes in this isolated variable may be approximated under these artificial conditions, but when studying the behavior of organisms in their natural environment, the variables can be segregated and evaluated only by statistical procedures. This entails a large number of observations or, as one of the authors has expressed it, "observations having a high degree of continuity" (Allen, 1934).

In a study of the distribution of phytoplankton the primary complicating factors are the cloud-like distribution of the plankton and the transport of these "clouds" by the water currents. At any one spot both the magnitude of the quantity and the specific composition may change completely within a few hours. Thus, at noon on December 27 at station 179, an anchor station of the 1940 cruise, there was a mean population of 2,000 diatoms per liter with *Pseudoeunotia* *doliolus* and *Guinardia flaccida* making up almost half of this number; at 7:30 the next morning a second series of samples revealed a jump in numbers of diatoms to 161,400 cells per liter with *Bacteriastrum* sp. and *Chaetoceros* sp. constituting over 90 per cent of this population. The need of both continuity of observations and a knowledge of the water movements is very great. In the Gulf of California the latter is of particular importance due to the presence of a standing internal wave which causes complex water movements, both horizontal and vertical of the upper water layers.

The foregoing remarks should not, however, be taken to imply in any way that series of limited numbers of samples have little or no value. Certainly they can and do add much to our knowledge of the region under consideration and though they do not in themselves lead to complete solutions, they point the way to such solutions.

REGIONAL DISTRIBUTION OF PHYTOPLANKTON

Allen (1937) and Cupp and Allen (1938) divided the Gulf into three geographical regions for convenience in comparing abundances of diatoms. The region south of 25° N. latitude was designated as the "southern section," the region between 25° N. and 27° N. latitudes as the "middle section," and the Gulf north of 27° N. latitude as the "northern section." In the present paper the "inner" shallow region of the Gulf north of 29° N. latitude is distinguished from the "outer" Gulf region south of this latitude. The previous divisions have been retained for the outer Gulf as these divisions correspond to the regions between the antinodes of the standing internal wave and, consequently, the water masses of these three regions are more or less segregated from one another.

TABLE I—PHYTOPLANKTON, 1939: MEAN NUMBER PER LITER

	Diatoms				
Station	*Good Condition*	*Poor Condition*	*Total*	*Dinoflagellates*	*Ast. japonica*
1	38200	2600	40800	40	67
2	3000	300	3300	20	
3	1200	100	1400	20	
4	4300	300	4600	40	
5	31800	2100	34000	80	
6	30300	2900	32200	20	
7	20900	2200	23000	60	
8	5900	1300	7200	660	
9	13500	2000	15600	80	
10	39100	8900	48000	840	
11	2300	300	2600	500	13
12	1400	300	1600	150	
13	25300	4600	29900	300	
14	30500	5800	36300	200	
16	20600	6400	27000	60	
17	1000	500	1600	80	
18	395500	47200	442700	100	1000
19	713000	117400	830400	200	1700
20	175700	66200	241900	170	250
21	1300	700	2000	90	
23	2600	1100	3700	20	
24	400	100	500	60	
25	300	200	500	60	
26	2400	500	2900	130	
27	1200	400	1600	100	
28	1000	300	1300	150	
29	4500	200	4600	130	
30	200	200	400	60	
31	1097000	86000	1183000	30	1028000
32	89900	155800	245700	220	156000
33	800	3700	4500	120	2100
34	1600	2000	3500	90	2000
35	73600	14200	87800	150	15200
36	3100	6400	9600	110	3200
37	60100	20500	80600	80	6500
38	6600	2300	8900	40	520
39	47600	9800	57500	140	4700
41	25600	1700	27300	1600	
42	10600	6600	17200	53600	
43	88900	35400	124300	36100	170
44	11700	4600	16300	33700	
45	97400	20800	118200	35300	
46	17500	14600	32100	34300	
47	21200	10800	32000	52900	
48	27900	5900	33800	29000	
49	1600	800	2400	46900	
50	79300	158400	237700	600	
51	4700	1600	6300	203400	
52	368200	60000	428200	19700	2900

TABLE I (cont.)

Station	*Nitz. seriata*	*Plank. sol*	*Pseud. doliolus*	*Rhiz. alata*	*Rhiz. bergonii*	*Thal. longissima*	*Gon. catanella*
1	11900	110	150	47	100	280	
2	710	73	50	5	32	37	
3	180	55	17	10	8	40	
4	190	200	370	37	15	150	
5	23100	540	200	40	500	420	
6	13800	420	1100	200	210	3100	
7	11200	180	380	37	120	520	
8	1200	1400	50	100	85	980	
9	1000	110	50	160	43	140	
10	5300	140	17	120	92	120	
11	510	77	33	58	10	1300	
12	600	100	83	68		320	
13	6200	770	1600	830	140	15600	
14	7200	410	580	510	70	13900	
16	3200	120	900	140		3800	
17	60	25	67	28	2	160	
18	6400	210	83	160		340	
19	9500	300	250			180	
20	330	260	1100	27		130	
21	300	25	180			5	
23	13	7	8			3	
24	47	8	110			3	
25	45	8					
26	270	20	100		2	7	
27	360	22	73			3	
28	130	7	130	2		3	
29	98	15	100				
30	8	100	20			12	
31	8700	37	170				
32	3400	7	530	33			
33	420		33	7		20	
34	330	13	100				
35	5800	27	170			7	
36	3600	40	380	7			
37	5400	7	180				
38	780	17	82				
39	6400	13	100				
41	1200		7	13		7	1600
42	330		240				53400
43	330		980				35900
44	25		25				33700
45	5300		2100				35000
46	1700	7	1600				33700
47	1400		1600			33	52200
48	1300		2100				27600
49	200						45300
50	73100		13		34		390
51	870		33				201300
52	5800		330				19700

TABLE I (cont.)

Station	*Ch. atlanticus*	*Ch. compressus*	*Ch. debilis*	*Ch. peruvianus*	*Ch. radicans*	*Ch. vanheurckii*	*Cosc. wailsii*
1	4500	950	1200	340	1500	550	
2	190	13		25	43		
3	210					47	
4	1100			53			
5	4200			250	17		
6	2200		50	90	470		
7	1400	33	47	140	1900	710	
8	350	220	200	43	17	100	
9	67	130	940	27			
10	150	3200	280	63			
11	73			48			
12	10			33	55	33	
13	170	50	100	340	100	180	
14	520		120	160	1100	220	
16	830	450	330	50	6200	400	
17	73	50		7	50	100	
18	150	14300	1600	7	139000	191000	
19	1900	25000	11200		27100	265000	
20	310	14000	1600	7	8600	87000	
21	95	17	270		33	130	30
23	7	17			42	33	3100
24	28					33	110
25	15					33	310
26	28	33	200			960	25
27	13		170			83	55
28		50	60			42	38
29			210		3500	280	20
30							57
31		6800	43500		11600	15400	
32	20	14000	5100		7500	14900	
33		83	250				
34	55	470	620		180	67	
35	290	2200	12000		9600	49000	
36	96	3700	3400		4600	4600	
37		3700	5600		8700	6700	
38	63	450	1200		1800	500	
39	33	7600	15900		6500	4100	
41		33	2500		2100	6200	
42		13	3500		2800	650	33
43	36	850	17000		46500	9200	17
44			4600		2300	980	
45		580	32900		6500	24500	
46	50	1100	6700		1900	1300	
47		100	5900		2600	1500	
48	180	170	530		14700	2000	
49			750				
50			117000				
51			3400				
52		24700	84500		128000	47700	

TABLE II—PHYTOPLANKTON. 1940

Station	Diatoms			Dinoflagellates
	Good Condition	Poor Condition	Total	
6	700	1100	1800	
9	900	700	1600	10
23	400	200	600	13
62	5300	200	5500	7
103*	31600	21200	53800	
105*	52400	12800	65200	
106*	23200	15400	38600	
108*	18900	2900	21800	
109*	97200	37800	135000	
110	200	100	400	
141	28400	4100	32500	
150*	29900	6100	36000	200
152*	20100	5000	25100	100
153*	98750	25600	124400	380
158*	5400	600	5900	50
175	154100	38400	192500	
178*	2300	640	2900	
179a	1700	400	2100	
179b	137400	24000	161400	
179c	107000	46800	153800	
188	1500	500	1900	
206	32000	3600	35600	
222	59300	13000	72300	
227	100	200	300	

* Designates surface sample. All other stations are represented by mean populations from zero to sixty meters depth.

SOUTHERN SECTION OF THE OUTER GULF

The previous expeditions to the Gulf had found this region to be characterized by the lowest phytoplankton populations of the entire Gulf. The only catches of any considerable size found in this region were made in 1921 off Espirtu Santo Island where catches of over 100,000 cells per liter were obtained in both April and June. A few populations of 10,000 cells per liter were also found in 1937 off Gorda Point, San Josef Island, and Mazatlan.

In 1939 two lines of stations, 1 through 17, were occupied in this region. While the populations found were not exceptional from a quantitative point of view, they were larger on the whole than had been reported from it previously. All stations showed mean populations of at least 1,000 cells per liter and at 9 of the 16 stations the mean number of cells per liter exceeded 10,000.

In 1940 only two stations were occupied in this region; station 6 (Oct. 12) and station 227 (Dec. 14). The mean diatom populations at these two stations were 1,800 and 300 cells per liter respectively.

In 1939 the plankton flora of this region, stations 1 through 17, could readily be distinguished from that of the other regions by its predominantly oceanic character. In general, *Nitzschia seriata* was the dominant species, but true oceanic forms were consistently present and constituted a considerable portion of the phytoplankton. These oceanic forms included *Chaetoceros atlanticus*, *Ch. peruvianus*, *Thalassiothrix longissima*, *Planktoniella sol*, *Rhizosolenia alata*, *Rh. bergonii*, *Rh. calcar-avis*, and *Rh. imbricata* var. *shrubsolei*. In the other sections of the Gulf these diatoms were either completely absent or were found only in a few scattered instances.

At stations 13 and 14 *Thalassiothrix longissima* was the dominant species and occurred in numbers up to 30,000 cells per liter to constitute more than half of the phytoplankton populations of these stations. This is interesting because a microscopic examination of the diatomaceous mud at the bottom of the Gulf revealed great quantities of the broken frustules of this diatom. It is one of the three forms that make up the main portion of the identifiable diatom frustules found in the Gulf sediments. The other two forms are *Coscinodiscus* sp. and *Pseudoeunotia doliolus*. In this respect it is of interest to note that *P. doliolus* was also present in considerable numbers and while this species was found at practically every station in the Gulf, it was most numerous in this southern portion and in the northern region.

MIDDLE SECTION OF THE OUTER GULF

This region, 25° N. latitude to 27° N. latitude, had been previously found to have diatom populations larger than those of the southern section but still relatively poor as compared with the more northerly regions of the Gulf. In 1921 half of the 18 samples taken in this region contained over 10,000 diatoms per liter and at several stations around Carmen Island populations of over 100,000 cells per liter were found. However, in the series of 1935, 1936, and 1937 the only samples of large populations came from off Topolabampa Bay where a catch of over 500,000 cells per liter was obtained in 1937 and from Conception Bay where populations over 10,000 cells per liter were found in both 1936 and 1937.

In 1939 huge catches of diatoms were made in the southwestern portion of this region. Stations 18 and 19 had mean populations of 442,700 and 830,400 cells per liter respectively, and station 20 had a mean population of over 200,000 cells per liter. However, the populations at the other stations of the regions were all under 5,000 cells per liter so that with the exception of the extremely large abundances in the southwestern corner, the phytoplankton of this region was the poorest found in the Gulf in 1939.

The large phytoplankton assemblages at stations 18, 19, and 20 were composed largely of neritic *Chaetoceros* species,—*Ch. radicans, Ch. vanheurckii, Ch. socialis, Ch. compressus*, and *Ch. curvisetus*. These neritic *Chaetoceros* species were common throughout the Gulf and usually made up the major part of the larger populations. The phytoplankton of the remaining stations of this region was clearly distinguished from that of the rest of the Gulf by the presence of moderately large numbers of a *Coscinodiscus* form either identical with or a variety of *Coscinodiscus wailsii* Gran and Angst.*

With the exception of stations 42 and 43, this diatom was not found outside of this middle region. The absence of this form at stations 18, 19, and 20 and the presence at these stations of *Asterionella japonica*, the characteristic diatom of the adjacent northern section, suggests strongly that the upper water mass at these three stations had drifted into this middle region from the adjacent northern section.

In 1940 eight surface samples were taken in this region. Six of these had populations greater than 10,000 cells per liter and one, station 153, at the mouth of Conception Bay, had a population of over 100,000 cells per liter. *Hemiaulus hauckii, Nitzschia seriata*, and the usually neritic *Chaetoceros* species dominated these samples. *Coscinodiscus wailsii* was not found at this time.

NORTHERN SECTION OF THE OUTER GULF

This portion of the outer Gulf, from 27° N. latitude to 29° N. latitude, includes the Guaymas area in which catches of over three million cells per liter had been made in 1937. Populations of the same size were again found here in 1939. In 1937 the main constituents of this population were *Chaetoceros radicans, Ch. compressus, Ch. debilis, Asterionella japonica*, and *Skeletonema costatum*. In 1939 the samples from this area, stations 31 and 32, were almost a pure culture of *Asterionella japonica*. Station 31 for example had a surface population of four million diatoms per liter, of which 3,600,000 were *Asterionella japonica*. In 1939 this species was almost entirely confined to this northern section of the outer Gulf and served to distinguish the flora of this region from that of the rest of the Gulf.

In 1939, of the ten stations in this area, five had mean populations of over 50,000 cells per liter. The phytoplankton of this region was distinctly more abundant than that of the more southerly regions.

---

* This diatom was identical to Gran and Angst's *Coscindodiscus wailsii* (Gran and Angst, 1931) except for the beginning of a rosette at the center, irregularly placed spinulae on the valve surface, and two small thickened processes on the valve flange at an angle of about 120° apart.

Station 30, the poorest of the section, evidently belonged to the adjacent middle section as *Coscinodiscus wailsii* was found here, whereas *Asterionella japonica* was missing.

In 1940 two stations, 175 and 179, of the ten occupied in this region had populations of over 100,000 cells per liter and two others, stations 141 and 222, had populations of over 30,000 cells per liter. The dominant species were neritic *Chaetoceros* species and *Bacteriastrum* species. *Asterionella japonica* was not found, but at station 188 a few species of *Coscinodiscus wailsii* were recorded.

INNER GULF REGION

In 1921 large diatom populations were found around Angel de la Guardia and Tiburon Islands and in the region to the north of these islands. In 1937 the region to the north of the islands was found to be very poor in phytoplankton, but exceptionally large diatom populations were found around the islands. From these series some suggestion was obtained that the area off the north end of Angel de la Guardia Island and the area off the southeastern portion of Tiburon Island were especially productive.

In 1939 these two areas off the islands were not visited, but in the rest of the inner Gulf region a completely different picture was obtained due to the presence of enormous numbers of the dinoflagellate, *Goniaulax catanella* (Whedon and Kofoid) or some species closely resembling it. Stations 41 and 50 were the only stations of this region that did not have large populations of this organism.

Diatoms were also present in large numbers in 1939. Ten of the twelve stations of this region had mean diatom populations of over 15,000 cells per liter and of these ten the mean populations of stations 43, 50, and 52 were over 100,000 diatoms per liter.

VERTICAL DISTRIBUTION

Our data are too few and our knowledge of the hydrographical conditions too vague to permit any serious attack on the problems of the vertical distribution of the phytoplankton,—especially in view of the fact that at a depth of 60 meters periodical vertical displacements of the order of 30 meters would be expected to accompany the internal wave.

In 1939 the depths of maximum populations of diatoms in good condition were distributed as follows:

0 meters — 9 stations
10 meters — 12 stations
20 meters — 9 stations
30 meters — 5 stations
40 meters — 6 stations
50 meters — 4 stations
60 meters — 5 stations.

In counting the organisms a distinction was made between the cells in good condition and those in poor condition (frustules lacking chromatophores or obviously disorganized) as the percentages of these two often aid in interpreting the results of the counts. It is interesting in this respect to note that there was no correlation between the percentage of cells in poor condition and the depth of maximum population. Nor was there any definite relation between the depth of the maximum number of cells in good condition and the depth of the maximum number of cells in poor condition—the maximum number of cells in poor condition being found as often above as below the depth of the maximum number of cells in good condition—which is in agreement with the hypothesis that the density of the cell protoplasm is close to or equal to that of sea water.

TABLE III—Vertical Distribution of Diatoms at Deep Stations, 1940

Station	*Depth in Meters*	*Good Condition*	*Poor Condition*	*Total*
9	0	480	1600	2080
	10	0	560	560
	20	20	460	480
	30	520	2000	2520
	40	1760	120	1880
	50	200	100	300
	60	60	40	100
	100	100	110	210
	300	0	65	65
	500	0	15	15
	750	0	50	50
	1250	0	5	5
	1500	5	0	5
	1620	0	10	10
23	0	140	80	220
	10	0	220	220
	20	20	160	180
	30	120	360	480
	40	760	60	820
	50	1720	80	1800
	60	0	120	120
	500	0	10	10
	750	0	40	40

TABLE III (Cont.)

Station	*Depth in Meters*	*Good Condition*	*Poor Condition*	*Total*
62	0	11520	320	11840
	10	6240	80	6320
	20	12000	440	12440
	30	2880	160	3040
	40	1440	280	1720
	50	3600	40	3640
	60	320	260	580
	500	0	40	40
	1525	0	10	10
110	0	1840	160	2000
	10	0	120	120
	20	100	40	140
	30	140	40	180
	40	140	140	280
	50	180	100	280
	60	40	220	260
	500	20	20	40
	1000	0	160	160
	1260	0	50	50
	1510	0	25	25
141	0	38400	6240	44640
	10	30400	3840	34240
	20	48160	15160	63320
	30	41760	10560	52320
	40	25440	10080	35520
	50	5440	3120	8560
	60	640	480	1120
	300	60	380	440
	500	240	350	590
	600	20	110	130
	760	70	40	110
	1000	70	170	240
	1740	10	100	110
175	0	336600	34800	371400
	10	178800	39000	217800
	20	293400	58200	351600
	30	181200	57000	238200
	40	79800	21200	101000
	50	20000	6560	26560
	60	7200	7520	14720
	300	240	820	1060
	500	0	400	400
	600	320	400	720
	750	220	750	970
	900	5	45	50
	1000	400	3740	4140
	1710	520	6800	7320
	1850	28	532	560
	1780	0	196	196
	1880	252	1540	1792

TABLE III (Cont.)

Station	*Depth in Meters*	*Good Condition*	*Poor Condition*	*Total*
179	0	333600	45600	379200
	10	313200	51000	364200
	20	270600	40200	310800
	30	62600	17200	79800
	40	9520	5040	14560
	50	1720	2080	3800
	60	320	1000	1320
	775	200	1520	1720
	860	240	640	880
	1020	0	280	280
	1200	0	1360	1360
	1020	40	100	140
188	0	320	140	460
	10	540	540	1080
	20	920	340	1260
	30	2360	440	2800
	40	1520	280	1800
	50	1400	540	1940
	60	3760	960	4720
	300	0	3560	3560
	480	222	4662	4884
	530	110	1375	1485
	580	0	495	495
	630	55	1705	1760
	670	330	3245	3575
	750	0	15000	15000
227	0	0	200	200
	10	160	240	400
	20	320	680	1000
	30	60	100	160
	40	60	20	80
	50	20	100	120
	60	80	20	100
	1000	20	20	40
	2050	60	140	200

Nine of the 1940 phytoplankton stations are of special interest in that at these stations samples were taken down to great depths; at stations 188 and 23 samples were taken down to 750 meters, and at stations 9, 62, 110, 141, 175, 179, and 188 down to depths below 1000 meters. As would be expected the number of empty frustules greatly exceeded the number of living cells at depths below 60 meters, and there was usually a sharp decrease in the number of total frustules between this depth and 300 meters, followed by a more gradual decrease below 300 meters, so that at 750 meters only a few hundred, or at the most one or two thousand, cells per liter were found. The one exception to this was station 188 where 15,000 frustules were found at 750 meters (the greatest depth sampled) whereas populations of only a few thousand cells per liter were found in the upper layers.

The specific composition changed considerably with depth. The majority of the weakly silicified, neritic diatoms such as *Chaetoceros radicans* and *Bacteriastrum* species disappeared between 60 and 300 meters and at 1000 meters very few representatives of these diatoms were found. The heavily silicified forms which were rarely present in large quantities in the surface layers increased, therefore, relatively with depth so that below 1000 meters the empty frustules of *Coscinodiscus*, *Thalassiothrix*, *Thallassionema*, *Pseudoeunotia*, etc. completely dominated the small residue of the diatom population.

The lists of species recorded for diatoms (table IV) and dinoflagellates (table V) are essentially the same as those recorded in other reports (e. g. Cupp and Allen, 1938), no significant differences being discernible.

TABLE IV—DIATOMS FOUND IN THE GULF OF CALIFORNIA IN 1939 AND 1940

Actinoptychus undulatus (Bail.) Ralfs	**Asterolampra marylandica* Ehr.
Asteromphalus heptactis (Breb.) Ralfs	*Asterionella japonica* Cl.
Bacteriastrum sp.	*Biddulphia* sp.
Chaetoceros affinis Laud.	*Ch. atlanticus* Cl.
Ch. compressus Laud.	*Ch. curvisetus* Cl.
Ch. debilis Cl.	**Ch. diadema* (Ehr.) Gran & Angst
Ch. didymus Ehr.	*Ch. lacinisosus* Schütt
Ch. lorenzianum Grun.	**Ch. messanensis* Castr.
**Ch. pendulum* Karst.	*Ch. peruvianus* Brightw.
Ch. radicans Schütt	*Ch. socialis* Laud.
**Ch. vanheurckii* Gran	*Chaetoceros* sp.
**Climacodium fraunfeldianum* Grun.	
Corethron criophilum Castr.	*Cor. hystrix* Cl.
**Coscinodiscus wailesii* (?) Gran & Angst	*Coscinodiscus* sp.
**Coscinosira polychorda* Gran	
**Dactyliosolen antarcticus* Cast.	*Dac. mediterranea* Perag.
Ditylum brightwellii (West) Grun.	*Eucampia zoodiacus* Ehr.
**Fragilaria* sp.	**Gossleriella tropica* Schütt
Guinardia flaccida (Castr.) Perag.	*Hemialus hauckii* Grun.
Lauderia borealis Gran	*Leptocylindricus danicus* Cl.
Lithodesmium undulatum Ehr.	**Licmophera lyngbyei* (Kutz.) Grun.
**Melosira* sp.	
**Navicula membranacea* Cl.	**Nav. pelagica* Cl.
Navicula sp.	
Nitzschia longissima (Breb.) Ralfs	*Nitz. seriata* Cl.
Nitz. pungens Cl.	

* Species not previously reported from the Gulf of California.

TABLE IV (cont.)

**Paralia sulcata* (Ehr.) Cl.
Planktoniella sol (Wall.) Schütt

Pleurosigma sp.
Pseudoeunotia doliolus (Wall.) Schütt

Rhizosolenia acuminata (Perag.) Gran
Rhiz. bergonii Perag.
Rhiz. castracanei Perag.
Rhiz. fragilissima Berg.
**Rhiz. robusta* Norm.
Rhiz. setigera Brightw.
Rhiz. alata Brightw.
Rhiz. calcar-avis Schultze
Rhiz. delicatula Cl.
Rhiz. imbricata Brightw.
Rhiz. hebetata f. *semispina* (Hensen) Gran
Rhiz. stolterfothii Perag.

Schroederella delicatula (Perag.) Pav.
Skeletonema costatum (Grev.) Cl.

**Stephanopyxis palmeriana* (Grev.) Grun.
**St. turris* (Grev. & Arn.) Ralfs

Surirella sp.

Thalassiosira decipiens (Grun.) Jorg.
**Thal. pacifica* Gran & Angst
**Thal. subtilis* (Ostf.) Gran
**Thal. gravida* Cl.
Thal. rotula Meun.

Thalassiothrix frauenfeldii Grun.
Th. longissima Cl. & Grun.
Th. hetermorpha Karst.

Thalassionema nitzschoides Grun.
**Tropidoneis* sp.

*Species not previously reported from the Gulf of California.

TABLE V—DINOFLAGELLATES FOUND IN THE GULF OF CALIFORNIA IN 1939 AND 1940

Ceratium candelabrum (Ehr.) Stein
Cer. fusus (Ehr.) Duj.
Cer. kofoidii Jörg.
Cer. tripos (O. F. M.) Nitzsch
Cer. furca (Ehr.) Clap. et Lachm.
Cer. macroceros (Ehr.) Cl.
Cer. pentagonum Gourret

Dinophysis caudata Saville-Kent
Din. ellipsoides Kof.

Goniaulax catanella Whedon & Kofoid
Goniaulax sp.
Gon. polyedra Stein

Noctiluca miliaris Sur

Peridinium crassipes Kof.
Per. divergens Ehr.
Per. orbiculare Paulsen
Per. pyriforme Paulsen
Per. steinii Jorg.
Per. depressum Bail.
Per. oceanicum Vanhöffen
Per. ovum Schiller
Per. pellucidum (Berg.) Schütt
Peridinium sp.

Phalacroma rotundatum (Clap. et Lachrm.) Kof. & Mich.
Podolampas palmipes Stein

Prorocentrum micans Ehr.

SEASONAL VARIATION

The productivity of the Gulf of California, so far as this is represented by the size of the phytoplankton populations, appears to be relatively great, but the data are far too deficient in continuity, both temporal and spatial, to permit any definite conclusions. Populations of over 100,000 cells per liter have been found at various stations throughout the Gulf in every season it has been sampled, but whether this would have been found to be the case by a continuous investigation extending over this period of the year, from November through June, is not known. Since three of the six cruises occurred in February and March, our knowledge of this period is more complete. In two of these three cruises huge populations were found, and it appears probable that this is the period of larger populations (a little earlier than in the Gulf of Santa Catalina).

The evidence for the annual phytoplankton cycle is largely indirect. Bottom cores from the Gulf show alternating light and dark bands which when examined under the microscope prove to be layers of relatively pure diatomaceous material alternating with layers of less purely diatomaceous detritus. These layers have been interpreted as annual layers corresponding to seasonal changes in diatom populations (Sverdrup and Staff, 1940) and if so indicate large seasonal differences in phytoplankton production.* In addition the waters are reported to be clear and blue in late summer but are discolored with phytoplankton growth over large areas in late winter and spring.

In the beginning of November strong northwesterly winds set in which must result in upwelling and replenishment of the nutrients in the surface layers. At the same time vertical circulation is brought about in the shallower upper region of the Gulf by the cooling of the surface waters with resulting lowering of the stability. These conditions should result in greatly accelerated phytoplankton production. In early summer the northwesterly winds die out; the surface waters become heated and stratified; and the phytoplankton should rapidly exhaust the supply of nutrients and diminish in quantity. This picture is partly confirmed by the fact that the largest populations have been found in March.

GENERAL CHARACTERISTICS OF THE GULF OF CALIFORNIA

The Gulf of California is a sloping trough, roughly V shaped in cross section, approximately 700 miles long and 60 miles wide. At the entrance its waters communicate freely with those of the Pacific Ocean down to a depth of more than 3,000 meters. The bottom slopes upward toward the Gulf's head and is irregular with several deeper basins present. About 29° N. latitude the Gulf becomes quite shallow and a group of islands rise above the surface. Beyond this point there are two deeper basins on each side of Angel de la Guardia Island but from them northward the depth is less than 200 meters.

---

* It should be borne in mind, however, that the diatoms whose frustules make up the diatomaceous deposits are not the same as those that make up the bulk of the phytoplankton pulses.

The temperature and salinity of the deep water in the outer Gulf show that this water is of the same type as exists over large areas in the Central Pacific. The general water circulation is characterized by an inflow of deep water and an outflow of surface water, and as a consequence of this circulation deep water, rich in plant nutrients, is brought to the euphotic zone where the nutrients can be utilized by the phytoplankton. The main factor effecting this circulation in the outer Gulf is the wind, which from late fall to summer blows parallel to and out of the Gulf, bringing about upwelling and an outward transport of surface water.

In the upper Gulf, i. e. in the region of and north of Angel de la Guardia and Tiburon Islands, there are two basins with sill depths of less than 200 fathoms in which temperature, salinity, and oxygen curves (Sverdrup, 1940) show that basin conditions exist. In winter, at the same time the northwesterly winds are bringing about upwelling in the outer Gulf, the surface temperature is decreased by cooling, and convection currents develop in these basins and in the shallow region to the north of the islands.

Thus in both inner and outer regions of the Gulf the hydrographical features are conducive to high productivity. These two conditions, upwelling in the outer basin and convection in the inner basin, can fully account for the fertility of the Gulf without the necessity of considering the effect of the Colorado River which empties into the head of the Gulf. That vertical oceanic circulation can maintain an enormous amount of marine life in the absence of local terrestrial drainage is clearly shown by the great productivity of the waters off the West Coast of South America which is one of the most arid coastal regions in the world.

At the time of the 1939 cruise salinity, temperature, oxygen, and density curves showed upward displacements suggestive of upwelling at stations 23, 39, and, to a lesser extent, 31. However, the hydrographical conditions in the outer Gulf in 1939 suggest the existence of a standing internal wave. Such a wave could give rise to similar displacements or could reduce the displacements due to upwelling, and caution must be exercised in utilizing such profiles to locate the centers of active upwelling.

The distribution of mass at the time of the 1939 cruise showed alternating upward and downward displacements of the isopycnal surfaces, and the dynamic computations revealed a sequence of separate highs and lows of the isobaric surfaces. This suggested the existence in the outer Gulf of a standing internal wave having three nodes (Sverdrup, 1940). The distribution of the mean diameter of the particles of the bottom sediments was found to agree with the presence of such a wave (Revelle, 1940) and suggested that this wave is a permanent feature of the Gulf. A theoretical treatment by Munk (1941) further showed that the postulated characteristics of the wave were compatible with the physical features of the Gulf. Additional confirmation for the existence of this wave now appears in the distribution of the phytoplankton.

The presence of such a standing internal wave with three nodes would be associated with three circulation cells in the outer Gulf whose boundaries would be located at the antinodes and a fourth cell comprising the area north of the island region where the wave terminates. This internal wave would set up horizontal cellular currents having maximum velocities of the order of 50 cm/sec but having zero velocity at the antinodes or cell boundaries. The direction of these currents in the middle cell would be opposite to those in the southern and northern cells and these directions would be reversed every half period, i. e. about 3.5 days for a first order wave or 7 days for a second order wave. In the case of waves with periods as great as these, the deflecting force of the earth's rotation would modify the direction of these currents so as to bring about a circular flow instead of a simple oscillation in one direction. This cellular circulation would act as a barrier to intercellular flow and would tend to preserve for a time any differences in the plankton flora of the cells.

In addition to this intracellular circulation there will be tendencies for intercellular circulation due to alterations in the distribution of mass brought about by the tangential stress of the wind and thermohaline changes in density. This will result in the frequent breakdown of the intercellular barriers and the transport of water masses across the antinodal regions. Any floral differences reflecting the presence of the internal wave will, therefore, be transitory in nature.

This internal wave greatly complicates the interpretation of the hydrographical data. As was mentioned previously, profiles of the physical properties of the water showed a large upward displacement at station 23. Such an upward displacement usually indicates upwelling and a consequent rich supply of nutrients in the surface layers. Yet this region was found to be extremely poor in phytoplankton. This raises the question as to whether the upward displacements of the isolines really indicated active upwelling. It is quite possible that this distribution of properties reflects the presence of the internal wave rather than upwelling. An anti-cyclonic circulation in this middle cell would be accompanied by upward displacements of the sigma-t surfaces along the shores and a downward displacement in the center; a condition which would simulate upwelling. This distribution of density would be reversed in the next half period when the anti-cyclonic circulation changed to a cyclonic one and any upwelling in this area would then be masked. Since the circulation in the adjacent cells is opposite in direction to that in the middle cell, the latter appears to be the case off Guaymas where a slight upward displacement of the isopycnals was accompanied by an extremely large diatom population.

On the other hand, it is equally possible, in the absence of any data and neglecting any intrinsic factors preventing the diatoms from responding to an increase in nutrients, to attribute the low populations of the middle region to zooplankton grazing. Also, other seasons of the year, or other years, may show differences in conditions.

SUMMARY

The examination of the distribution of the phytoplankton in the Gulf of California in 1939 disclosed four distinct phytoplankton types; an oceanic type at stations 1 through 17, a *Coscinodiscus wailsii* type at stations 21 through 30, an *Asterionella japonica* type at stations 31 through 39, and a *Goniaulax catanella* type at stations 41 through 52. The distribution of these types corresponds exactly to the locations of the circulation cells which would be predicted in the presence of a standing internal wave as postulated by Sverdrup and constitutes confirmative evidence for the existence of this wave.

In agreement with the results of previous cruises to the Gulf, the phytoplankton populations of the northern sections of the Gulf were found to be larger on the whole than those from the southern section. This indicates that upwelling is most active in the northern section of the outer Gulf (the Guaymas area) where slight upward displacements of the isopycnal surfaces were found in spite of an opposing tendency of the distribution of mass accompanying the internal wave.

Indirect evidence indicates that the seasonal phytoplankton cycle is dependent upon the process of upwelling in the outer Gulf and upon convection in the upper, shallow region. These two processes are initiated in late fall when northwesterly winds bring about upwelling and when surface cooling lowers the stability of the water masses in the basins and shallow areas of the upper Gulf. At the onset of summer when the northwesterly winds die out and the surface waters become heated and stratified, these processes and the spring phytoplankton growth terminate. The fact that the largest populations have been found in March supports this picture and suggests that this is the period of maximum productivity.

Phytoplankton samples from depths greater than 300 meters were obtained in 1940 and indicate that the majority of the weakly silicified cells were dissolved above this depth and that the numbers of frustules cleansed of cell contents greatly exceeded the number of cells in apparently good condition.

REFERENCES

Allen, W. E.

1923. Observations on surface distribution of marine diatoms of Lower California in 1921. Proc. Cal. Acad. Sci. *12:* 437–42.

1934. The importance of continuity in marine plankton investigations. Fifth Pac. Sci. Cong. California, 1933, Proc. *3:* 2051–55.

1937. Plankton diatoms of the Gulf of California obtained by the G. Allan Hancock Expedition of 1936. The Hancock Pacific Expeditions, The Univ. So. Calif. Publ. *3:* 47–59.

1938. The Templeton Crocker Expedition to the Gulf of California in 1935—the Phytoplankton. Trans. Amer. Micr. Soc. *67:* 328–35.

Bigelow, H. B., Lillick, L. C., Sears, M.

1941. Phytoplankton and plankton protozoa of the offshore waters of the Gulf of Maine. Part I—Numerical Distribution. Trans. Amer. Phil. Soc. N. S., *31:* 149–91.

Cupp, E. E. and Allen, W. E.

1938. Plankton diatoms of the Gulf of California obtained by Allan Hancock Pacific Expedition of 1937. The Hancock Pacific Expeditions, The Univ. So. Calif. Publ. *3:* 61–74.

Gran, H. H. and Angst, E. C.

1931. Plankton diatoms of Puget Sound. Publ. Puget Sound Biol. Sta. *7:* 417–519.

Munk, W. H.

1941. Internal waves in the Gulf of California. Sears Foun., J. Mar. Res. *4:* 81–91.

Revelle, R. R.

1939. Sediments of the Gulf of California. Geol. Soc. Amer., Bull. *50:* 170–71.

Sverdrup, H. U.

1941. The Gulf of California: Preliminary discussion of the cruise of the "E. W. Scripps" in February and March, 1939. Sixth Pac. Sci. Cong., Calif., 1939, Proc. *3:* 161–66.

Whedon, W. F. and Kofoid, C. A.

1936. Dinoflagellata of the San Francisco Region. I. On the skeletal morphology of two new species, *Gonyiaulax catanella* and *G. acatanella.* Univ. Cal. Pub. Zool. *41:* 25–34.

Contribution No. 184

Reprinted from ARCHIVES OF BIOCHEMISTRY
Vol. 1, No. 3, February, 1943

Adenochrome, a Glandular Pigment in the Branchial Hearts of the Octopus[1]

Denis L. Fox and David M. Updegraff

From the Scripps Institution of Oceanography of the University of California, La Jolla, California

Received October 20, 1942

A recent publication from this laboratory (1) which dealt chiefly with the carotenoids in the digestive gland of the two-spotted octopus, *Paroctopus bimaculatus*, gave passing reference to a blood-red, water-soluble pigment encountered in the "kidneys" of this species. Actually, the structures in which the pigment occurs are the branchial hearts, a pair of small, purple, oval-shaped pulsating organs, situated outside of the large kidney-sac proper, one at the base of each gill. These branchial hearts are partly muscular, serving to pump blood into the gills, but composed largely of spongy, glandular tissue, supposedly of excretory function. In the glandular cells are stored considerable quantities of the pigment in the form of deep garnet-red cell-inclusions. The properties of this pigment and its deposition exclusively in tissue of supposed excretory role lead us to believe it to be a metabolic waste product (2–7).

PHYSICAL PROPERTIES AND CHEMICAL BEHAVIOR OF THE PIGMENT

A. *Appearance, color and solubility:* The name adenochrome which we have given to this pigment arose from the fact that its only locus of deposition seems to be in the glandular tissues of the branchial hearts. Here it shows a strikingly uniform distribution throughout the component cells (see Fig. 1), imparting a purplish color to the whole organs.

Fresh sections placed upon microscope slides yielded their pigment slowly to water, rapidly to dilute ammonia, leaving in each cell one or more colorless granular masses which formerly incorporated the colored material.

---

[1] Contributions from the Scripps Institution of Oceanography, New Series No. 184.

Adenochrome is fairly soluble in water, especially if warm, yielding wine-red colloidal solutions which manifest a Tyndall beam and from which the pigment fails to diffuse through commercial animal-membranes, even against a gradient of saturated NaCl solution. It is not precipitated from neutral aqueous solutions by boiling or by saturation with NaCl or $(NH_4)_2SO_4$. It is readily soluble in alkali at all concentrations.

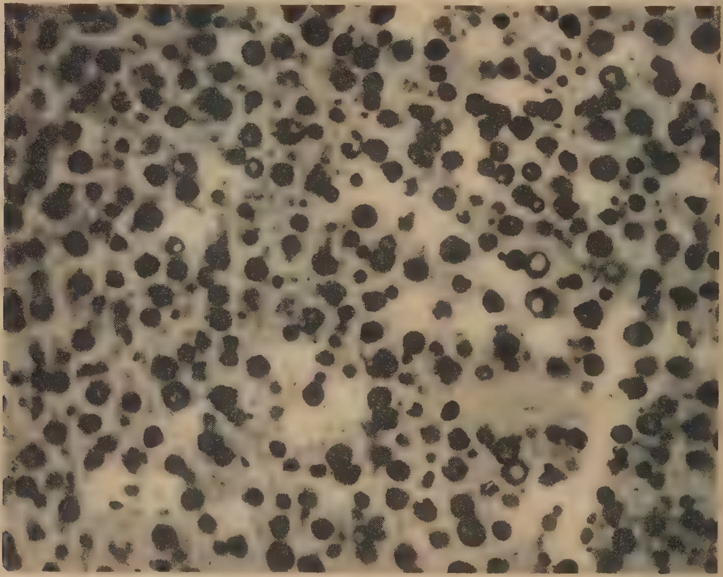


FIG. 1. Pigment inclusions in glandular cells of branchial heart tissue. (× ca. 270)

The addition of dilute acid or of small amounts of alcohol or acetone to a dilute solution of the pigment causes a change from wine-red color to a purplish, turbid appearance, and results in the eventual precipitation of the pigment in amorphous flocks.

Appreciable concentrations of alcohol (30% to 50% by volume) bring about the ready and complete precipitation of adenochrome, if a trace of acid is added. Neutral solutions require greater amounts of the precipitant, and alkaline solutions have failed to yield a precipitate by the addition of alcohol. These observations suggested the weakly acidic nature of adenochrome, which was later confirmed by titration with dilute alkali.

B. *Preparation and yields of pigment:* On the basis of the solubility of adenochrome in water (ca. 0.7 g. per l.) and aqueous systems and its insolubility in alcohol, a simple procedure was achieved for its isolation in nearly quantitative amounts. From weighed specimens sacrificed by chilling in a refrigerator, the branchial hearts were carefully removed, weighed and extracted either wet or following drying *in vacuo* over $CaCl_2$ at room temperatures. (Oven-drying at 108°C. was found to result in partial decomposition, giving a somewhat charred residue.) The mass was then ground in dilute NH_4OH or Na_2CO_3 solution (approx. 0.5%) until fine comminution of the material had resulted. The aqueous extract was decanted and the remaining solid material re-extracted a few times until it assumed a pale color and yielded no more pigment to the dilute alkali. Rendering the combined extract faintly acidic by the addition of dilute acetic acid was useful in promoting the complete coagulation of all protein material by boiling at this stage. The system was then cooled, rendered faintly alkaline again with dilute NH_4OH or Na_2CO_3, and filtered from the coagulum through paper. The filtrate was rendered barely acidic with dilute acetic acid, treated with an equal volume of 95% ethanol, and the resulting purple flocks of pigment were separated by centrifugation. Washing of the precipitated material with 50 to 75% ethanol, and sometimes with absolute ethanol and with ether, was followed by redissolution in dilute alkali, acidification with dilute acetic acid, and a second precipitation with one-third the volume of alcohol. Avoidance of the use of strong alcohol and of drying the material precluded the formation of a water-insoluble component, which could, however, be rendered soluble again by taking up in dilute base, acidifying slightly, and precipitating in 30% alcohol without now permitting the substance to dry out. The final method of isolation adopted therefore avoided the use of alcohol stronger than 30% (final concentration); furthermore, neither tissues nor pigment were dried unless this was necessary for analysis. The final dry weight was determined by evaporating aliquot portions of the pigment solution to dryness at 80° to 90°C., and the ash values were readily obtained from such residues.

Table I furnishes representative data regarding the proportional weights of the branchial hearts, and the yield of adenochrome stored therein. It will be noted that the glands themselves are very small, their wet weight constituting only from 0.13 to 0.47% of that of the whole body, the average value being about 0.23%. There appears to be no correlation with age (weight) or sex. The presence of blood in the removed glands could hardly have introduced a variation greater than about 0.06 g., but this possible factor has not been seriously considered, since the isolated glands often continued to pulsate without visible bleeding.

Taking the average dry weight as 26.5% for grouped specimens Nos. 6 to 9 and employing the *final* weight of recovered adenochrome (after a second precipitation, save in the case of specimen No. 5, wherein this step was omitted), we arrived at a figure of 23% as the approximate mean yield of pigment from dry gland, the extremes being 11% and 37%.

TABLE I

Proportional Weights of Branchial Hearts; Yields of Pigment

Octopus No. (sex)	Wet body wt.	Wet wt. of branchial hearts (% of total)	Dry wt. of branchial hearts (% dry wt.)	Wt. pigment, 1st pptn. (% yielded)	Wt pigment, 2nd pptn. (% yielded)
	g.	g.	g.	g.	g.
1 (♂)	791	2.343 (0.29%)	0.712 (30.3%)	0.300 (42.1%)	0.263 (37.0%)
2 (♀)	1116.5	3.29 (0.295%)	0.91 (27.6%)	0.250 (27.4%)	0.196 (21.6%)
3 (♂)	103.7	0.244 (0.235%)			
4 (♀)	288.0	0.495 (0.17%)	0.126 (25.4%)	0.018 (14.3%)	0.014* (11.1%)
5 (♀)	382.6	0.494 (0.13%)	0.132 (26.6%)	0.022 (16.7%)	
6 (♂)	348.6	0.519 (0.15%)			
7 (♂)	263.0	0.554 (0.21%)			0.071 (3.28% of wet, or ca. 12.4% of dry)
8 (♀)	272.5	0.382 (0.14%)			
9 (♀)	489.0	0.706 (0.14%)			
10 (♂)	189.2	0.885 (0.47%)			

* Low figure; slight loss of pigment occurred.

C. *Spectrum and chemical properties:* Adenochrome, prepared in the dry state, is an amorphous mauve or purple powder with slightly hygroscopic properties. It shows no visible evidence of decomposition at temperatures up to 300°C., and has no melting point, but chars readily at higher temperatures.

Besides its insolubility in alcohol or acetone, and its precipitation thereby from neutral or acidified aqueous solutions, adenochrome fails to dissolve in ether, chloroform, dioxane, glacial acetic acid, and most other organic solvents. It was found to be soluble in pure fused trichloracetic acid, diluted pyridine, and slightly in hot glycerol.

Aqueous solutions of the pigment showed relatively low tinctorial power. Therefore, in order to observe the absorption spectrum, care was exercised to obtain a relatively strong aqueous solution, free from excessive optical scattering. Although a minor Tyndall beam was always present, dilute NH_4OH or Na_2CO_3 served to minimize scattering which otherwise obliterated the broad maximum observed between about 500 to 520 mμ.

A Bausch and Lomb visual spectrophotometer was used in direct observation of the absorption maximum of adenochrome in dilute alkali. In Curve A, Fig. 2, the extinction coefficient *e* is plotted against wave length of emergent light. The formula is employed as follows:

$$e = \frac{\log_{10} \frac{I_0}{I}}{cL}$$

where $\log_{10} \frac{I_o}{I} = d$ or Density units measured directly by the instrument.

I_o = The light transmitted by solvent alone, and
I = That transmitted by the pigment system,
c = Concentration of pigment in grams per liter,
L = Length of column of solution in centimeters.

In curve *B*, *d* of a solution of pigment in a relatively high state of purification but of unknown concentration is plotted against wave length. The data serve to confirm the shape of curve *A*, and to sharpen the region of maximum absorption in the broad, smooth region involved. Our pigment shows a maximum at about 505 mμ.

The color of the pigment itself, and color-changes produced by reactions with certain reagents were useful in studying some further properties of adenochrome. Following are some of its reactions.

Oxidizing agents

Concentrated HNO_3:	Bright orange color.
Dilute $KMnO_4$:	Brown red, bleaching to brown yellow (unlike yellow of urochrome so treated).
Withdrawal of O_2:	No change.
Concentrated H_2SO_4:	Brown color, deepening by charring.
Bromine:	Dark amorphous precipitate.

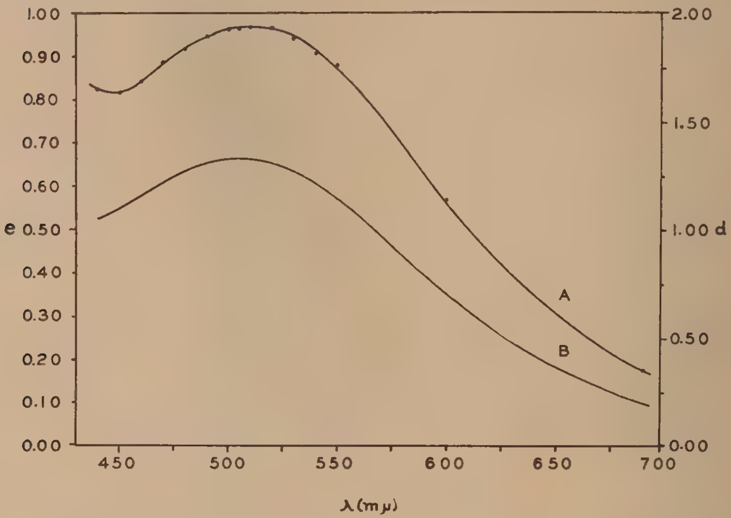


FIG. 2. Absorption spectrum of adenochrome in water containing traces of alkali Curve *A*: Wave length plotted against absorption coefficient, *e* (left). Curve *B*: Wave length plotted against density, *d* (right).

Reducing agents

Zinc dust + KOH:	Rapid decolorization.
Na_2S:	No immediate reaction, but boiling changed pink color to pale brown.
$Na_2S_2O_4$:	Bleached to pale yellow color, reversible by aeration.
$NaHSO_3$:	No change.

Adsorbents

Talc:	No adsorption.	
Kaolin:	No adsorption.	
Al_2O_3:	No adsorption.	
Starch:	No adsorption.	
MgO:	Strong adsorption	Probably because of pigments' acidic properties, hence its ability to form salts.
$Ca(OH)_2$:	Strong adsorption	

Specific tests

Salkowski's, for indole, pyrrole:	Negative, with raw pigment or hydrolyzates.
Pine-splinter + HCl for pyrrole:	Positive, from roasting pigment.
Murexide test for purines:	Negative.
Schiff's, for aldehydes:	Negative.
Liebermann's, for monohydroxy phenols:	Negative.
Benedict's for reducing sugars:	Negative on acid hydrolyzate (no carbohydrate material).
Lipoids:	Absent, shown by insolubility in ordinary lipoid solvents, solubility in aqueous systems.
Phosphorus:	Absent, shown by negative reaction with molybdate + oxidized digest.
Halogens:	Absent, shown by negative reaction with $AgNO_3$ + digest.

Protein reactions. A (adenochrome similar)

Roasting:	Familiar odor of burning hair or feathers.
Ninhydrin reaction:	Strong (α-amino groups, or certain alkyl amines).
Biuret reaction:	Very faint.
Diffusion through animal membranes:	No.
Precipitation with dialyzed iron:	Quantitative.
Precipitation with $FeCl_3$:	Yes.
Precipitation with Ag^+:	Yes.
Precipitation with Cu^{++}:	Yes.
Precipitation with Pb^{++}:	Yes.
Precipitation with Hg^{++}:	Yes.
Precipitation with phosphotungstate:	Yes.
Precipitation with phosphomolybdate:	Yes.
Precipitation with picrate:	Yes.

Protein reactions. B (adenochrome dissimilar)

Boiling water or dilute acid:	No coagulum.
Alcohol or acetone:	Precipitate redissolved readily in dilute alkali.
Xanthoproteic reaction:	Negative.
Millon's test:	Negative.
Aldehyde test for tryptophan:	Negative.
Sakaguchi reaction for arginine:	Negative.
Reduced sulfur:	Absent (all S in oxidized state).
Trichloroacetic acid:	Dissolved powdered pigment (precipitates proteins).

The alkaline and the acid hydrolyzates of adenochrome failed to show any of the principal reactions not manifested by the original pigment.

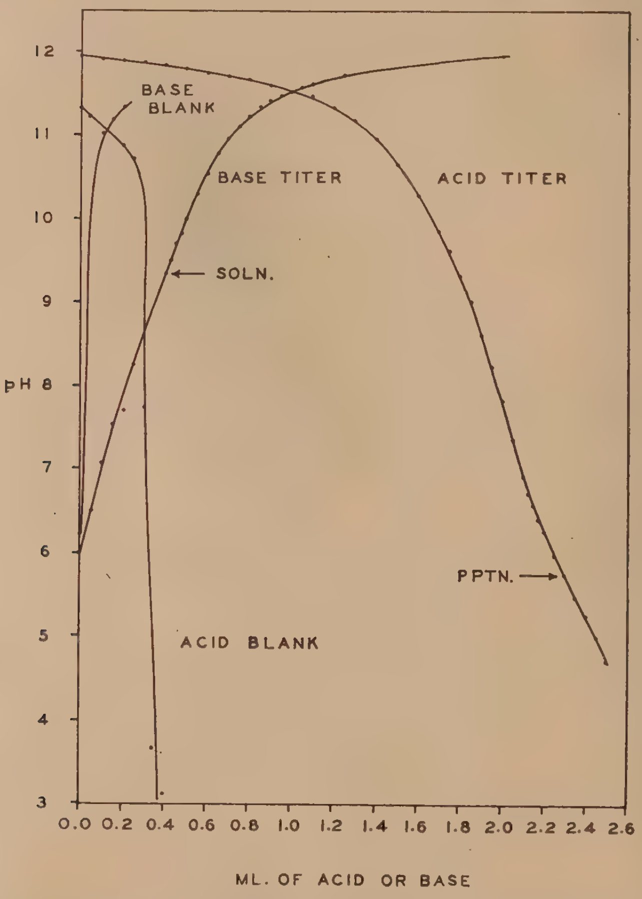


Fig. 3. Characteristic titration curves for adenochrome
Initial concentrations: 0.0775 g. adenochrome, partly dissolved, partly suspended in 10 ml. H_2O; titrated directly with 0.306 *N* NaOH to pH 11.95; back titrated with 0.26 *N* HCl, using a Beckmann glass electrode and pH meter.

Although adenochrome bears a few resemblances to protein, certain striking dissimilarities exclude it from identification with that class of compounds.

Attempts were made to gain an idea of the equivalent weight of adenochrome by titrating it with standard alkali, using the Beckmann glass electrode and pH meter calibrated with the use of a standard solution of *d*-glutamic acid. Fig. 3 shows that, while adenochrome possesses considerable buffering capacity as a weak acid, its titration curve is quite smooth throughout, the absence of a break making it impossible to determine the true point of neutralization. Our adenochrome-in-water system showed an initial pH value of about 5.97. The particulate phase disappeared at about pH 9.35, giving way to the familiar colloidal condition. During back-titration with standard acid, wherein a smooth curve of similar shape resulted, reprecipitation of the pigment as red-purple flocks took place near pH 5.75.

The low solubility of our product, and the resulting necessity of working with very dilute solutions (save when in the distinctly alkaline phase), are contributing factors to the difficulty of establishing a range of neutralization in titration curves. The initial slightly acidic condition of adenochrome in water, and the gradual increase in pH with added alkali, in contrast to the rapid rise shown by the control, would seem to characterize the pigment as a compound containing a number of weakly acidic groups. Since phenols are absent, these acidic groups are probably carboxyl, perhaps accompanied by one or more sulfoxy bodies per molecule.

ELEMENTARY COMPOSITION

Qualitative analytical tests showed the presence of C, H, N, S (oxidized state) and ash containing Fe. Through the kindness of Dr. A. J. Haagen-Smit at the California Institute of Technology, two different preparations of adenochrome were dried in high vacuum at 50°C. for 30 minutes and subjected to quantitative microanalysis for C, H, N and ash, and one sample was analyzed for S. The pigment's insolubility in camphor precluded a molecular weight determination.

Carefully and repeatedly purified preparations of pigment samples showed considerable variation in ash (*i.e.*, from 4.2 to 7.4%) and in total iron (0.43 to 0.67%). Iron was determined either in ashed samples by the colorimetric method of Wong (8) or without preliminary ashing by a slight modification of his method, giving identical results by both procedures.

Iron seems to be present chiefly in the ferric state, from which it is reduced by the pigment in acidic systems. It is possible that we may be dealing with a ferric compound associated with the larger adenochrome molecule through a partial neutralization of colloidal charges. In this connection dialyzed iron, a colloid carrying a positive charge, is readily adsorbed to adenochrome and brings about mutual precipitation if added in sufficient quantity. Ferric chloride has a like effect.

TABLE II

Elementary Micro-analysis of Adenochrome (Kindness of Dr. A. J. Haagen-Smit and Dr. G. Oppenheimer)

	mg. sample	mg. CO_2	% C	mg. H_2O	% H
Preparation A					
Sample 1	3.833	5.514	39.26	1.827	5.33
Sample 2	3.035	4.505	40.51	1.446	5.33
		ml. N_2	% N		
Sample 1	3.603	0.423 (27°C.; 744 mm.)	12.78		
Sample 2	2.339	0.243 (26°C.; 744 mm.)	11.33		
		mg. ash	% ash		
Sample 1	3.833	0.188	4.90		
Sample 2	3.035	0.152	5.00		
		mg. CO_2	% C		
Preparation B					
Sample 1	3.205	4.428	37.70	1.663	5.81
Sample 2	2.532	3.496	37.68	1.352	5.97
		ml. N_2	% N		
Sample 1	3.090	0.392 (26°C.; 741 mm.)	13.78		
Sample 2	2.876	0.368 (25°C.; 741 mm.)	13.93		
		mg. ash	% ash	mg. $BaSO_4$	% S
Sample 1	4.233	0.308	7.28	1.664	5.40
Sample 2	5.687	0.423	7.44	2.172	5.24

Iron constitutes but a minute portion of the pigment, and only from one tenth to one eighth of the ash, in which were detected no alkali metals, alkaline earths nor any traces of copper (by diethyl-dithiocarbamate test). Most other heavy metals were also ruled out by the failure of H_2S to yield precipitates in iron-free acidic solutions of the ash. However, a faint dark turbidity appeared in iron-free ammoniacal solutions of the ash in the presence of H_2S. This suggested possible traces of nickel or cobalt, but our small supply of material did not allow further investigation of these trace-metals.

Because iron was present in only minute quantities, inconstant in relative amounts, easily and quantitatively split away from the pigment and representing only a small fraction of the total ash, we concluded that it may be associated with the adenochrome molecule only in an incidental way, and not stoichiometrically. The change in color of the pigment from red or pink to pale blue or green by the addition of acids was reversed by alkali, although the traces of iron were dissociated from the pigment by treatment with acids, and probably remained so.

TABLE III

Composition of Adenochrome

	1. (Average of microanalyses)	2. (Ash-free basis)	3. (Per cent composition/atomic weight)	4. (Data of column 3, multiplied by 12, as least common multiple to give approximate whole numbers)
	per cent	*per cent*	*per cent*	
C	38.79	41.33	3.44	41.33
H	5.61	5.98	5.98	71.76
N	12.955	13.80	0.985	11.82
S	5.32	5.67	0.177	2.12
O	31.17*	33.22	2.076	24.91
Ash	6.155			
	100.000	100.00		

* By difference.

Table II shows figures which yield grand average values for the elementary composition of adenochrome shown in Table III (Column 1). In the light of the above considerations regarding the disposition of the small amounts of iron, we recalculated the composition of the pigment on an ash-free basis, obtaining the results shown in Column 2. Column

3 shows the quotient of the percentage of each element divided by its atomic weight. In order to arrive at a provisional empirical formula for adenochrome, it is necessary to show at least one atom of sulfur. Multiplying the values in Column 3 by 6, however, while giving 1.06 atom of sulfur, yields fractional values for those of carbon (20.66) and oxygen (12.45). We have therefore used the next possible multiplier, 12, with results shown in Column 4 which are probably better approximations to the real values.

While the calculated numbers of constituent atoms do not emerge as whole numbers, there is seen to be at least a fair degree of approximation. Furthermore, our observations and the microanalytical results would indicate that adenochrome is not a substance which is easy, or even possible to prepare in absolutely pure form, just as the complex urinary pigment urochrome and the widely distributed black pigment melanin have never been so prepared. Our provisional empirical formula for adenochrome, based on the above data, would appear to be approximately according to the constitution $C_{41}H_{72}N_{12}O_{25}S_2$, with a minimum molecular weight of about 1200.

State of Sulfur

On numerous occasions isolated preparations of the pigment were treated with boiling alkali, and the digest subsequently tested for reduced sulfur with lead acetate. Even after prolonged treatment with concentrated NaOH, no traces of PbS were ever obtained; thus the possible presence of cystine, or of any other compounds containing —S-S— or —SH groups were eliminated. Prolonged boiling in concentrated alkali or fusion in $Na_2CO_3 + KNO_3$ mixture yielded SO_4^{--}, however, precipitable as the barium salt which could be dissolved in concentrated H_2SO_4 and reprecipitated by dilution. Although sulfur was thus demonstrated to be present in oxidized form, its position in the pigment was not determined. Maintaining the pigment in the presence of $Ba(NO_3)_2$ in neutral, acidic, or alkaline solution for several hours at 100°C. failed to bring about the precipitation of $BaSO_4$, although the pigment underwent some decomposition or precipitation. This would indicate that the oxidized sulphur was held in a stable position.

Qualitative Distribution of Nitrogen

Solutions of adenochrome so dilute as to show no color alone gave a blue violet color with triketohydrindene hydrate. Use was made of the adaptation of this color reaction by Harding and MacLean (9) for the determination of α-amino nitrogen, although the reaction is also given by ethyl amine, trimethyl amine and benzyl amine.

Instead of comparing our solutions directly with a standard reference compound in a colorimeter, we measured the light absorption of solutions at 571 mμ, the absorption maximum, with the use of the Bausch and Lomb visual spectrophotometer. For comparison, a standard solution of Eastman's *d,l*-alanine was used. By this method adenochrome was estimated to contain quantities of α-amino nitrogen (or alkyl amines) corresponding to 2.88%.

Effervescence of very small bubbles was observed when adenochrome (even when prepared by extraction with 1% Na_2CO_3 instead of dilute ammonia) was treated with cold concentrated NaOH or KOH. Moist litmus paper suspended above such systems soon turned blue in color. Accordingly, amide or ammonium nitrogen was suspected. A small quantity (0.0692 g.) of the pigment was treated with ca. 8% KOH (initial concentration) in a Kjeldahl flask, and the contents were boiled gently, the distillate being collected in dilute standardized HCl. When volatile base ceased to appear in the distillate, aliquot samples were estimated for ammonia by nesslerization, using a standard $(NH_4)_2SO_4$ solution for comparison. Aliquots were also titrated with dilute standardized NaOH using methyl orange as indicator.

A slight loss of distillate doubtless rendered somewhat low the nesslerization figure of 4%, and gave a high value to the titration figure of 7.7%. However, the apparent amide nitrogen value lay between these limits and was assumed to represent ammonia, although we might have been dealing with alkyl amines or with mixtures of volatile bases.

Following the distillation of all volatile base, further boiling with the now very concentrated KOH caused the distillation of traces of a non-basic substance of very foul odor, reminiscent of fecal compounds. It gave no test for indole or pyrrole with *p*-dimethylaminobenzaldehyde, but the addition of phenyl diazonium sulfonate gave a weakly positive reaction indicative of imidazoles.

Fresh adenochrome solutions, as well as the distillation-residue from the above experiment gave consistently negative results with Millon's test for phenols; the phenol test with $FeCl_3$ was inconclusive. Positive reactions for the imidazole nucleus were always obtained with the Ehrlich diazo reagent (sulfanilic acid in dilute HCl or HNO_3; dilute $NaNO_2$). The yellow color which finally developed from an initial orange was similar to that of histidine treated in like manner, which goes through a preliminary red phase.

Therefore, the procedure described by Snell (10; pp. 219 *et seq.*) was employed, using aliquot portions of the adenochrome distillation-residue and a standardized dilute solution of histidine, each diazotized and matched spectrophotometrically at 440 mμ (the maximum point of absorption). Our results indicated that roughly 2 to 3% of the pigment existed as imidazole nitrogen. The imidazole configuration in adenochrome was concluded not to be histidine or histamine, since neither of these compounds yielded a substance in their distillates which gave a positive reaction with Ehrlich's reagent.

Chemical comparisons show some resemblances and some striking differences between adenochrome and urochrome, the yellow urinary pigment of mammals.

The extensive crustacean factor in the normal diet of the octopus suggested also a comparison of adenochrome with Verne's amino acid pigment from the hypodermis of crustaceans.

Table IV presents a resumé of such properties of adenochrome as render possible a comparison with urochrome, described by Verne (11) and Dombrowski (12), and with Verne's crustacean pigment (13).

The comparison, while revealing the numerous differences between adenochrome and urochrome, brings out a sufficient number of similarities to suggest the possibility of some metabolic parallelism in the elaboration of the two pigments in widely separated phyla. The known properties of Verne's crustacean pigment are too few for an extensive comparison, but the substance is clearly different from adenochrome. Verne reports positive xanthoproteic and Millon's reactions with his crustacean pigment, but is not certain that a protein contaminant might not be responsible.

Adenochrome occupies a rather unique position from the standpoint of both biochemistry and physiology. Our studies would suggest that the pigment is a nitrogenous waste product. This is indicated especially by the apparent storage of much nitrogen in an amide state, releasable as free ammonia in the presence of strong alkali; the pigment thus may act as a kind of reservoir to maintain catabolic ammonia and/or perhaps alkyl amines (giving the ninhydrin reaction), beneath the toxic threshold. Again, the presence of considerable imidazole nitrogen suggests that we may be dealing with an excretory substance analogous to the waste product, urochrome. The presence of oxidized

TABLE IV

Comparison Between Adenochrome, Urochrome, and Verne's Amino Acid Pigment

	Adenochrome	Urochrome	Verne's amino acid pigment
1. Source	Branchial hearts of octopus	Urine of mammals (increased in fever, etc.)	Chromatophores of crustacean hypodermis.
2. Color	Red in solution; amorphous mauve to purple powder when dry.	Yellow in solution; amorphous brown powder when dry.	Yellow.
3. Composition	C 41.33% H 5.98 N 13.80 S 5.67 O 33.22 (Fe traces)	C 43.42% H 5.32 N 10.78 S 5.9 O 34.58	—
4 Solubilities	Sl. sol. H_2O; v. sl. sol. dil. acids; v. sol. alkalis; sol. strong acids; sl. sol. trichloracetic acid; sol. dil. pyridine; insol. 30 to 100% alc., acetone, ether, chloroform, glacial acetic a., CS_2, pure pyridine, cold glycerol. Slightly hygroscopic.	V. sol. H_2O; sol. acids and alkalis easily altered thereby; sl. sol. alc.; insol. ether, benzene, chloroform. Very hygroscopic.	Sol. cold H_2O; v. sol. hot H_2O; sol. dil. acids and bases, trichloracetic acid; insol. alc. and fat solvents.
5. Reduced sulfur	Absent; all S in oxidized form.	Present.	Present.
6. Pauly's diazo reaction	Positive.	Positive.	—
7. Pyrrole test	Positive.	Positive.	—
8. Murexide test	Negative.	—	Negative.

TABLE IV—*Concluded*

	Adenochrome	Urochrome	Verne's amino acid pigment
9. Acid hydrolysis	6 to 8 hrs. with 33% H_2SO_4 gave little brown sediment; principal chemical reactions unchanged.	8 hrs. with ca. 6 *N* HCl gave uromelanin quantitatively.	—
10. Free NH_3	Readily evolved.	—	Not detected.
11. Protein precipitants	Pptd. by Ag^+ in alk. soln.; Cu^{++} in neut. soln.; Pb^{++}, Fe^{+++} in aq. soln's, and Hg^{++} in acid or neut. soln.; by phosphotungstic, phosphomolybdic or picric acids.	Pptd. from aq. soln's by Cu^{++}, Hg^{++}, Pb^{++}, or Fe^{+++}; also by phosphotungstic or phosphomolybdic acid.	Not pptd. by ammoniacal $AgNO_3$ or by $CuSO_4$ after treatment with $NaHSO_3$.
12. Weiss or Heflebower test for urochrome or urochromogen	Dil. $KMnO_4$ → brownish color.	Dil. $KMnO_4$ → increased yellow color.	—
13. Thudichum's test for urochrome.	$Hg(NO_3)_2$ → red-brown pptn., unchanged by boiling.	$Hg(NO_3)_2$ → white pptn., boiling → rose red.	—

sulfur in the pigment may be indicative of the operation of detoxicating mechanisms similar to those which take place in mammalian tissues, involving the sulfonation of certain poisonous molecules.

The pigment is stored solely in the glandular tissue of the two branchial hearts, whose major blood supply has first passed through that region of the vena cava which is invested with the excretory nephridial gland. Should the nephridial gland constitute a channel for the gradual excretion of adenochrome, the presence of this pigment in the gland would have been expected, but such was never observed to be the case.

Nor was any evidence found for the possible elimination of the pigment from the gill tissues. Indeed any efficient manner of elimination of the pigment was difficult to imagine, since adenochrome is an acidic substance, soluble in faintly alkaline media, whereas the pH of fresh branchial heart tissue was from 4.9 to 5.04 (Beckmann glass electrode) and hence afforded an unfavorable medium for the pigment's dissolution or hence its transportation.

We have not been able to decide upon the metabolic origin or fate of adenochrome. It is hoped that future investigations may be more revealing in this regard.

Acknowledgments: We take pleasure in expressing our appreciation and thanks to the following: Dr. Wesley R. Coe, Professor Emeritus of Zoology, Yale University, for his friendly interest, useful advice and generous assistance; Dr. A. J. Haagen-Smit, in charge of the microanalytical laboratory at the California Institute of Technology, and Dr. G. Oppenheimer, microanalyst in the same laboratory, for the elementary analyses of our material, and Professor L. Zechmeister at the California Institute of Technology for his continued friendly interest.

SUMMARY

1. The glandular walls of the gill-hearts in the octopus *Paroctopus bimaculatus* contain relatively large quantities of a red, intracellular acidic pigment, adenochrome, which is readily extracted with dilute alkalies to give wine-red colloidal solutions with a single absorption-maximum in the visible spectrum at about 505 $m\mu$.

2. Adenochrome is insoluble in ordinary fat-solvents including alcohol and acetone, either of which precipitate it from neutral or slightly acidic aqueous systems as amorphous flocks, readily resoluble in dilute alkali. The purified pigment shows no melting point, but decomposes above 300°.

3. Readily oxidized by concentrated nitric or sulfuric acids, $KMnO_4$, or bromine, adenochrome is also readily reduced by zinc dust and alkali, warm Na_2S, and reversibly by hydrosulfite.

4. The new pigment fails to show several critical reactions of proteins, and exhibits some types of behavior not encountered in proteins. It was estimated to contain nearly 3% of α-amino nitrogen and/or alkyl amines, some 4 to 7% of amide nitrogen, and comparable amounts of imidazole nitrogen. Pyrrole bodies were demonstrable only by roasting. Neither purines nor phenols were detected.

5. The ash content varied between about 4 and 7.4%, invariably

containing traces of iron, besides possibly cobalt or nickel, but no copper, alkali metals or alkaline earths. The iron seemed to be present only incidentally.

6. From microanalytical data, the minimum empirical formula for adenochrome has been calculated on an ash-free basis as $C_{41}H_{72}N_{12}O_{25}S_2$, with a minimum molecular weight of about 1200.

7. The elementary constitution of adenochrome is similar to that of urochrome, but the two compounds differ in many respects, notably in disposition of sulfur which is entirely in the oxidized condition in adenochrome, but yielded in the reduced state by urochrome.

8. The possible position of adenochrome as an excretory substance is discussed briefly.

References

1. Fox, D. L., and Crane, S. C., *Biol. Bull.* **82,** 284 (1942).
2. Isgrove, Annie, Eledone (The Octopod Cuttlefish). *L.M.B.C. Memoirs,* No. 18, *Proc. Trans. Liverpool Biological Society* **23,** 469–574 (1909).
3. Cuenot, L., *Arch. Zoöl. Exptl.* (2) **9,** 19 (1891).
4. Harless, E., *Archiv Naturgeschichte* **13,** 1 (1847).
5. Vigelius, W. J., *Niederl. Arch. Zoöl.,* **5,** 115 (1880).
6. Kruckenberg, C. Fr. W., *Untersuch. aus dem Physiol. Inst. Heidelberg,* **2,** 402 (1882).
7. von Fürth, O., *Z. physiol. Chem.* **31,** 353 (1900).
8. Wong, S. Y., *J. Biol. Chem.* **77,** 409 (1928).
9. Harding, V. J., and MacLean, R. M., *J. Biol. Chem.* **20,** 217 (1915).
10. Snell, F. D., and Snell, C. T., Colorimetric Methods of Analysis, vol. 2, Organic and Biological, Van Nostrand, New York (1937).
11. Verne, J., Les Pigments dans l'Organisme Animal, G. Doin et Cie., Paris (1926).
12. Dombrowski, G. B., *Z. physiol. Chem.* **62** (1909).
13. Verne, J., Pigments tegumentaires des Crustaces decapodes. Arch. de Morphologie, Gen. et Exptl., G. Doin, Paris (1923).

Contribution No. 186

New Series, No. 186

Shoreline Erosion at La Jolla, Ca

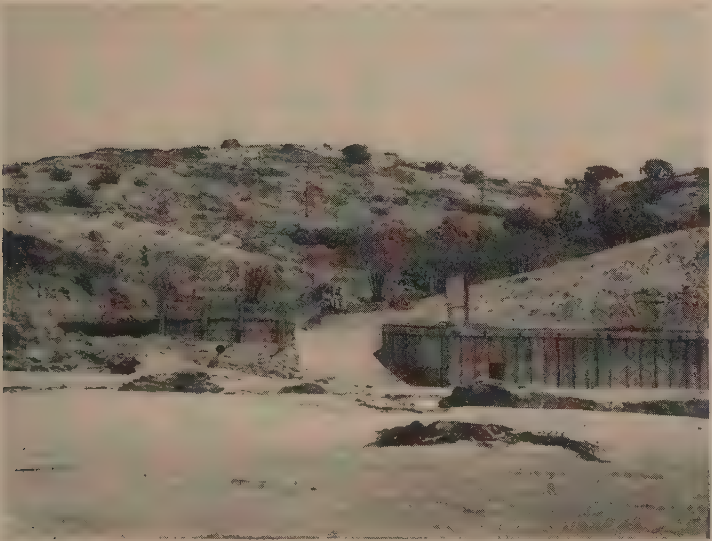
Special Instruments Developed in Study of Factors Responsible for Slow Rece

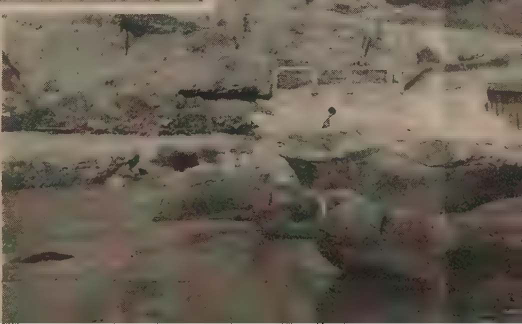
By Francis P. Shepard
University of California, San Diego, Calif.

Moderately resistant rock formations and the relative infrequency of severe storms along the coast of Southern California are the factors accounting for the slow rate of recession of the sea cliffs at La Jolla. Records of weather conditions, currents, and movements of beach materials over a period of years have assisted greatly in a determination of the factors conducive to rapid erosion. Dr. Shepard contributes this paper, originally presented before the San Diego Section, as a result of his work with the Scripps Institution of Oceanography, La Jolla, Calif.

An occasional landslide or collapse of a sea arch has led some residents of La Jolla and other coastal towns of Southern California to believe that the rock cliffs are retreating at an alarming rate. However, examination of a large number of photographs taken from 20 to 45 years ago has shown that there is almost no indication of important wave erosion during that period. Most of the changes that have occurred might be expected as the result of the weathering and erosion of these sea cliffs by rain storms. In many places projecting concretionary knobs on the cliffs make identification very certain. Few of these knobs have been removed.

Photographs by Dr. U. S. Grant IV and the writer, taken along other portions of the coast of Southern California indicate, for the most part, the same absence of erosion of consolidated formations. It is impossible to say whether the absence of recent erosion of these rocky sea cliffs is due to the failure of any major storm to strike them in recent years, but this seems a reasonable hypothesis. The sea cliffs may





Above, Beach Sand at a High Level at the End of a Quiet Summer; Right, Rock Formations Exposed After Winter Storms

Varying Beach Levels at the Scripps Institution, La Jolla, Calif.

could have be
present rate o
dence indicate
not been stabl
period to pro
present rates o

As for the se
formations of
much less stal
where such dep
erosion is obse
at a comparati
erosion is ordi
stormy winter
when the beac
away leaving
base of the cliffs. In some cases the
removed, exposing the underlying f
removal of the sand berm, the cliffs n
in the course of one stormy season
that which hit the New England
1938 should attack the Southern Ca
of a score or more feet co
the soft formations, and i
tant retreats would also
rocky cliffs.

In a study of the factor
shoreline erosion in this
weather, waves, current,
variables were begun at
Since that time many thou
have been made of the lev
the pier of the Scripps Ins
raphy at La Jolla, and alo
to the south of it. Since
reference points have be
day. In conjunction with

at the end of the pier have been
re-recording machine. Also, cur-
ed almost continuously for about
rrent meter. Over all this period
ne Coast and Geodetic Survey and
ve been obtained from the same

ation of the local beach, notes
e to the gravel cover, the char-

NE PROVIDES DAILY DATA AT THE
S INSTITUTION PIER

the abundance of shells and kelp.
en obtained periodically from both
area and these have been analyzed
For comparative purposes ob-
been made of the sand heights at
orth and Crystal Pier to the south.
ave been taken to determine the
urrent circulation in La Jolla Bay.
ff erosion in the La Jolla area has
easurement and by comparison

of a cooperative nature. Much of
or directed by E. C. LaFond, who
ng with Carl Johnson, for designing
d the pier sounding machine used.
director of the Scripps Institution
s co-designer with Ott Dahl of the
urrent meter and has made many
uring the course of the investiga-
study of the wave records has been
by Dr. W. F. Wohnus. Extensive
collected by Paul Williams. Vari-
ve contributed to the gathering of
ilation of data. This brief paper is
ndertakings of various observers at
n, of whom the writer has been one.

MPLOYED IN THE INVESTIGATION

ment used in these studies was de-
this work, and so far as the writer
used elsewhere. It may be of inter-
escriptions of these instruments and

ng machine is operated on the end
about 900 ft from shore, at a point
ays outside the breaker zone. By
es anchored to the ocean bottom, a
the pier to the surface of the ocean

FLOAT USED WITH THE WAVE-RECORDING MACHINE

in motion for about 15 minutes every day. It records the size of the waves and the periods between them, also the irregularity of the wave surface. These various characteristics are all important in connection with the eroding power of the waves.

Also located at the end of the pier is the Dahl-Sverdrup current meter, which operates electrically, making a continuous record of the direction and speed of the current moving along the coast, without, however, recording the offshore or onshore components of this current. This continuous record has shown that erroneous interpretations may result from occasional current readings. Although the currents are predominantly southerly in direction, north-moving currents are very common. The latter are observed almost every day, and may even predominate over the southerly currents for considerable periods of time. A study of these current records has shown that there is a direct relation between the currents and the beach erosion.

The sand-measuring machine is placed on the railing of the Scripps pier and rolled along it the length of the pier. Measurements are made at stations marked every 20 ft on both sides of the pier. The distance from the railing to the sand is read by the relation of markers on the wire to a yardstick along the top of the machine. By this means changes of as little as one-tenth of a foot are recorded, but changes of as much as several feet have been found during a 24-hour interval. The largest daily changes are most commonly excavations, but large fills occur during some of these short intervals.

Sand measurements along the beach south of the Institution pier require no instruments other than a measuring rod. A number of marks have been placed along sea walls and other structures usually reached by the waves at high tide. The height of these marks above the sand level is recorded each day. Other points out on the open beach are measured by means of ranges giving accurately located reference points, which are used to tell the height of the sand.

A fact that has been well-established by this work is that sand is carried seaward away from the beach during the stormy season of the winter and back onto the beach during the calm weather of summer. During some periods of large waves, the sand has been cut away from all 15 of the beach stations and along almost the entire length of the pier. When the waves were small, the sand was seen to build up onto the beach along almost its entire length. A continued period of such smooth seas causes the growth of a flat-topped berm along a beach where previously there had been a continuous outward slope of sand. As evidence that the sand does not move far from shore, it has been found that the sand level

Scripps Institution Pier, from Which Measurements of Factors Causing Beach Erosion Are Made

found near shore migrate outward during the stormy season and back again during the calm weather. There is some movement of sand along the shore, but this appears to be less significant than the movement normal to the shore.

At points along the beach where sea walls have been built in front of sea cliffs, there is much more change in the sand level than where a sea wall borders a low stretch of coast. Also, the down cutting in winter is much less pronounced inside the head of a submarine canyon where the waves are less pronounced.

The amount of cutting in a particular storm is dependent on (1) the height of the waves, (2) the period of the waves, (3) the height attained by the highest tides of the storm period, and (4) the steepness of the beach preceding the storm. Maximum cutting is done by storms with the largest waves of longest period (between crests), during the highest tides, and where the beaches have the steepest slopes.

In some cases when there were large waves during periods of low tidal range, the beaches next to the sea walls were actually filled instead of cut. Also, the times of most extensive cutting have come so clearly at times when there were waves of long period that there can be little doubt regarding the importance of this wave characteristic. It was found that long-period waves come from winter storms much more intense than ordinarily develop along the coast of Southern California. A study of the weather maps, by Dr. Sverdruk and others, has shown that such waves originate in an area that is commonly from 600 to 2,000 miles out to sea off the California coast. Observations of these storms by ships may possibly be used in the future to predict the development of destructive waves along the coast.

ACTION OF RIP CURRENTS

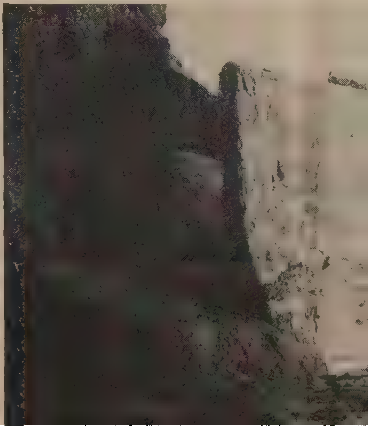
The effect of currents on beach erosion is manifested in several ways. A type of water movement out from

clearly related t
rent which coul
seaward at the
months. Thes
small channels r
moves seaward
as to cause c
bathers. The
by bathers prob
rents. Where
erosion, any mea
location would
to the erosion.
of rubble into t
might be expo
produce such a

The chief cu
end of the Scri
the south or t
the south-movin
common and th
winter. The c
lated to sea lev
daily by averag
of the tide. A
panied by a sou
a north-flowing
high sea level.

There is, furt
between mean sea level (and curre
sand, both on the beach and along
levels accompany low sand, and t
sand. Clearly the lowering of the
the cutting away of the sand, bu
which is no doubt caused by the l
some effect in cutting the sand.
of lowered sea level is the cooling
although it seems likely that ce
also have some bearing on this le
accompanies large waves even la
water has become considerably
temperatures.

These conclusions, based on
should prove of some value to e
problems of beach erosion. So
have no doubt already been antic
connection with their work, but c
been overlooked where there has
to make observations over a con



Contribution No. 187

Reprinted from SCIENCE, January 22, 1943, Vol. 97,
No. 2508, pages 89–90.

AGAR-BEARING SEAWEEDS AT LA JOLLA, CALIFORNIA[1]

THE use of red seaweeds as a source of jellies began and developed in the Far East. Nine tenths of the world's agar is produced in Japan by a purely cottage industry, while on the shores of China, Malaya, the East Indies and Ceylon, there is manufactured by native methods a jelly which, if dried, would be the agar of commerce. Of about thirty species of red algae which are recorded as agariferous, only four are used outside of the regions named. It is possible that on the southern and eastern shores of Asia all profitable sources of agar have already been found, but on the other coasts of the world there probably exist considerable resources of this kind which have never been developed. The prospect of competition with cheap Japanese agar has prevented large-scale investment in exploratory and developmental processes. In Russia, with government protection, there is a modern agar industry of less than ten years' standing, utilizing two species of seaweeds, *Ahnfeltia plicata* on the Maritime coast and in the White Sea and *Phyllophora rubens* in the Black Sea. In this country, repeated efforts during the last twenty years have finally established a modern manufacture utilizing *Gelidium cartilagineum* and *Gracilaria confervoides* collected on the coasts of southern California and Lower California.

With Oriental supplies of agar cut off, attention in this country has been turned to agar substitutes and the reclamation of agar, as recent notes in this journal testify. Believing with Humm[2] that, in addition, considerable domestic resources exist, both as unlocated beds of *Gelidium* and *Gracilaria* and as beds of red algae whose agar-producing potentialities have never been recognized, the authors have examined and tested several red algae growing at La Jolla in harvestable quantities. Restrictions on the use of boats at sea have delayed the extensive survey which the situation demands and limited us to collecting at low tide and by swimming and diving in shallow water. About two miles of exposed rocky shoreline have been covered and considerable quantities of agariferous seaweeds located.

---

[1] Contributions from the Scripps Institution of Oceanography, New Series, No. 187.

[2] H. J. Humm, SCIENCE, 96: 230–231, 1942.

The seaweeds were drained and weighed, freed of sand, shells and conspicuous contaminating species while being washed in tap water, dried in the sun and weighed again. Bleaching was not attempted. Fifty gram lots of dried material were weighed out and soaked in tap water for twenty-four hours except where otherwise noted. Each lot was drained and put into one liter of tap water. Sulfuric acid or sodium hydroxide was added to adjust the solution to pH 6.0, 8.0, 10.0 or 12.0, except as otherwise noted and the material cooked for twenty-four hours in a water bath. Distilled water was added periodically to replace evaporation losses. Every two hours a 25 ml sample was removed and cooled to 23°–25°. The strength of the jelly (if a jelly was formed) was measured with a Lipowitz meter as described by Kizevetter[3] in which a segment of a sphere of stated dimensions is driven through the surface of the jelly by weights. The pH was measured and, when necessary, acid or alkali was added to the parent lot to maintain the pH at the value fixed on initially. The cooled sample was stirred back into the parent lot. At the end of the single extraction a 100 gram sample was filtered through two layers of cheesecloth, cooled, frozen, chopped, thawed at room temperature, drained and air dried on a glass plate. The dried agar was weighed, and a 1 per cent. jelly was made from part of it for a strength test.

Six species were readily collected in sufficient quantities to test. *Gelidium cartilagineum* grows below the low tide mark and is collected commercially by divers working as deep as ten fathoms. However, fronds 50–75 cm long can be collected in quantity just below lower low tide. *Pterocladia sp.* grows low in the intertidal zone, the 12–18 cm fronds thickly covering exposed rocks. *Endocladia muricata,* 5–8 cm long, grows higher in the same zone. *Gigartina canaliculata,* with fronds 10–12 cm in length, also occurs in the intertidal zone. *Gigartina serrata* with fronds 20–30 cm. long grows below low tide, very densely in partially shaded places. *Gigartina asperifolia* was the only species collected which so far seems unsuitable as an agar source.

---

[3] I. V. Kizevetter, *Bull. Pac. Sci. Inst. Fisheries and Oceanography (Russian)*, 13: 1–135, 1937.

A year-round study will be necessary to establish the amounts of these seaweeds which, on the average, can be collected in a day, week or month and the annual harvest which can be expected from a section of coastline. In summer, with a calm sea, approximately 100 to 300 pounds of wet seaweed of any of the species named can be collected at one tide by our methods. The dry weight varies from 17 to 25 per cent. of the wet weight. The species growing near or below low tide are large and can be collected rapidly but only for a short time each day, while the species of the intertidal zone take more time to collect, but more time is available.

The yield of agar from *G. cartilagineum* was twice as great at pH 6.0 and pH 8.0 as at pH 10.0 and pH 12.0. In the case of one lot no acid or alkali was added and it maintained itself at pH 8.0 during the cooking. At pH 6.0 and pH 8.0 the strength of the jelly increased steadily during the first eighteen hours and afterwards remained unchanged until cooking was stopped after twenty-seven hours. This experience corresponds with the traditional Japanese method which includes the addition of a little vinegar to the cooking water and with the American commercial method in which no attempt is made to control the acidity.

The yield from *Pterocladia* was greatest at pH 6.0, but there was little difference between all lots except the lot at pH 10.0 which showed a very small yield. At pH 6.0 the maximum jelly strength was reached after fourteen hours cooking, after which a 20 per cent. decrease occurred. The greatest yield would probably have been obtained by stopping the extraction at pH 6.0 at the end of fourteen hours.

The yield of agar from *Endocladia muricata* was nearly twice as great at pH 12.0 as in any other lot.

The maximum jelly strength was reached after fourteen hours' cooking and remained constant until cooking stopped. The yield of dry matter from *E. muricata* was about twice as great as from *G. cartilagineum* and from *Pterocladia*. However, the firmness of a 1 per cent. jelly was only half as great. Either *Endocladia* agar has a low jellying power or the crude dry extract contains considerable impurities.

None of the three species of *Gigartina* tested yielded a jelly after fourteen hours cooking at pH 6.0, 8.0, 10.0 or 12.0. In a second experiment, the alkalinity of the different lots was maintained by cooking in tenth molar calcium chloride, with excess calcium carbonate, or with excess calcium hydroxide, respectively. Under these conditions *Gig. canaliculata* at pH 12.0 (excess calcium hydroxide) yielded a soft jelly. Following the method described by Kizevetter[3] for the treatment of *Ahnfeltia plicata,* dried seaweed of each of the three species was soaked for three days either (a) in cold saturated calcium hydroxide or (b) in cold 2 per cent. calcium chloride. *Gig. asperifolia* was so badly disintegrated by these treatments that it could not be handled. The other two species yielded firm jellies after two to five hours' cooking either in 2 per cent. calcium chloride or in saturated calcium hydroxide. Under these conditions, the two species of *Gigartina* act like *Gracilaria* in yielding their agar readily.

The most important information obtained is that out of five species of red algae not previously considered agariferous, harvestable in moderate quantity at La Jolla without special equipment, four readily yielded agar, in quantity and quality equal to that obtained from red algae at present commercially exploited.

ROBERT H. TSCHUDY
MARSTON C. SARGENT
SCRIPPS INSTITUTION OF OCEANOGRAPHY
OF THE UNIVERSITY OF CALIFORNIA,
LA JOLLA

Contribution No. 188

Reprinted from TURTOX NEWS, Vol. 21, No. 1, January, 1943.

MICROSCOPIC CREATURES AFLOAT IN WATER

by
W. E. Allen
Scripps Institution of Oceanography of the University of California

Contributions from the Scripps Institution of Oceanography
New Series, No. 188

The man who by his first sight of a giraffe was surprised into saying "There ain't no such animal" probably would be too much stupefied for any kind of expression if he could only see some of the microscopic creatures so commonly abounding near to his hand. Of these, some of the most astonishing are to be found afloat in water or swimming through it.

The invisible (or nearly invisible) animals and plants composing many submerged populations carried in suspension by widely differing aggregations or masses of water may be interesting to many kinds of people for many kinds of reasons. Most easily satisfied is the person who merely likes to get a thrill of astonishment by seeing a monstrous shape, the like of which never has been encountered in even the wildest of his dreams. Least easily satisfied, perhaps, is the person who wonders how an interesting specimen happened to be where it was caught, what the history of it and its kind might be, what its abundance might be or might become, what its relations to other creatures and to its surroundings might be, what peculiarities of existence it might have to show, what details of form and structure might be observable, etc., etc.

A drop of water is not too small in amount, and an ocean of water is not too large to contain many individual specimens of living things. On the other hand, one may find certain drops of "natural water" which appear to be barren and he may examine a large volume of water from certain parts of any ocean at certain times without finding many (if any) microscopic specimens. However, there are few drops of exposed water completely barren in the neighborhood of any human habitations.

It is commonly true that if one possesses a good microscope which will magnify as much as one hundred diameters he need not leave his place of abode to find good hunting for active specimens. Even his drinking water is likely to prove interesting, although he may have to strain (or condense) a considerable quantity (pints, quarts, or gallons) before finding good specimens sufficiently large to show with that magnification. As for door yard or roadside puddles remaining for a day or two (or more) after a rain, single drops often are teeming with tiny inhabitants.

The first discovery of such closeness of interesting neighbors was made by Leeuwenhoek (in Holland) in 1675, in rain water which had been standing "four days in a new earthen pot," glazed within. Apparently, it has been only about one hundred years (1845) since microscopic creatures were discovered in the sea, at least, since particular attention was given to them by Johannes Müller who found many interesting specimens captured by the nets being towed by him for catching larval echinoderms.

Methods of capture of microscopic inhabitants of water in use today are not fundamentally different from those used by Leeuwenhoek and Johannes Müller. On the one hand, examination is made of the populations in a particular volume of water. On the other hand, the populations are withdrawn from or separated from indefinite or definite quantities of water which may contain them. I, myself, have used methods ranging from one extreme to the other.

Years ago I noticed a pool of water with a purplish red color in the bed of a drying irrigation ditch. I dipped a sample into a small bottle and examined a drop under my microscope at home. It was an almost "pure culture" of *Euglena,* scores of individuals in every drop. Even in the ocean, I have dipped up samples which showed many specimens of microscopic creatures in each drop. In one period of three years (1912-1915) I collected "microplankton" (microscopic animals and plants) regularly from the San Joaquin river by drawing a fine meshed silk net through the water capturing the tiny organisms in a way not far different from that used by a fisherman when he dips a seine or hand net into the water to haul out some fish.

Since 1919 I have been concerned mainly with studies of populations of microscopic plants (phytoplankton) in sea water. In most years and through most of any year it is necessary to concentrate the included organisms of at least a liter of sea water if one desires to obtain a sample that is reasonably representative of populations whose individuals have dimensions ranging from one hundredth to eight tenths of a millimeter. Inasmuch as densities of populations have constituted the foundation of my investigations, I needed to know as dependably as possible the numbers of plants occurring in particular units of water.

My tow net catches in the San Joaquin River had been highly unsatisfactory because there was no way of knowing how much water was strained (filtered). One haul thirty meters in length might remove all of the desired organisms in a column of water of that length. Another haul at the same place on another day might remove less than one tenth of those present because of clogging or other errors. Because of this experience, I depended on measuring the amount of sea water sampled when I began operations at the Scripps Institution of Oceanography of the University of California.

Through the years 1920 to 1929, inclusive, daily catches were made at the Scripps Institution pier (and at another pier over one hundred miles distant) by the simple process of dipping up from the sea five gallons of water and straining (filtering) it through a small conical net of No. 25 silk bolting cloth. After 1929 the daily catches at the Institution pier were made by dipping up one liter of sea water, pouring it into a tall cylinder, adding formaldehyde (for killing and preserving) and allowing the contents to settle to the bottom.

Since 1926 all of thousands of catches of phytoplankton obtained by ships at sea have been made by dipping up water and straining five or ten liters of it through a small filtration net. For surface dipping from a ship running at regular speed off shore any kind of suitable dipper (usually a galvanized pail or a canvas bucket) has been used. For samples below the surface with a ship drifting or at anchor, a closing bottle (Allen Bottle) of five liters capacity has been used almost exclusively.

My studies of microscopic organisms in the San Joaquin River were intended to be quantitative, i.e., they were intended to reveal how population densities differed from week to week and season to season, and how species representation of microscopic animals and plants changed through the same periods. Crude as were the methods of collecting, it is true that they did reveal such characteristics of river populations to a remarkable extent. Still these methods were never satisfactory to me because I realized that they were lacking and probably misleading in respect to many details of population characteristics and environmental relationships. After finding so much success and satisfaction in my methods of collecting marine phytoplankton, I have wished often that I could repeat the river investigations with similar methods of operation.

Other investigators have originated and developed their own kinds of equipment and details of using them, but, so far as work in "fresh waters" is concerned it seems to be generally understood that no satisfactory quantitative researches can be made without examining the contents of known (measured) volumes of water. This is as it should be, of course. What seems to be less well understood and appreciated is the fact that anyone having access to a microscope of moderate magnifying power may obtain some of the thrills of Leeuwenhoek and other pioneers by merely dipping up samples of exposed water and by examining them directly or after suitable concentration.

I am sure that some people lose their opportunities for enjoying such thrills because they assume that serious difficulties must be surmounted before reaching them. But Leeuwenhoek was not deterred by the necessity for making an entire new microscope (very simple to be sure) for each individual specimen in many cases. It seems, also, that he never developed any method of concentrating his samples. Not only are our microscopes (even the poorer ones) far superior to his but we have the advantage of knowing that water apparently barren may be made to yield a catch by straining (filtering) small or larger quantities of it.

We have, also, the advantage of wider and more numerous incentives. Whereas his observations were stimulated mainly by his desire to discover new wonders in the form of living creatures, plus his desire to test each new microscope that he made, we may go far beyond this to complicated studies of microscopic populations as supporters of fish populations or as ultimate sources of much of our own food or other supplies. Indeed, we can go so far as to investigate the importance of these creatures in modifying carbon dioxide and other chemical constitution of waters, their values as indicators of movements of water masses and other physical conditions, and of their influence on obstruction of light in water.

The bad taste or smell (or both) often noticed in our city water supplies should be sufficient reminder of the potency of chemical influences of some "fresh water" plankton organisms, some of which are involved ordinarily.

Perhaps enough has been said to indicate that interesting microscopic neighbors abound all around us and that many of them can be observed with little effort almost any day. If one wishes to be sure to have something to examine, he may follow Leeuwenhoek's example in making a little preliminary preparation. After making his original discovery in the drop of rain water stagnating for four days he soon began trying out various combinations of leaves, etc., which he soaked in water. Perhaps some of us have noticed a whitish, jelly-like scum about the stems of flowers left in a jar of water two or three days longer than intended. Doubtless, he found many of his wonders in scum like this.

For those of us who wish to be more systematic in study of the microscopic populations in water it is necessary to examine quantities known at least approximately. A certain measured volume of water must be taken and the included organisms must be counted or measured. So far as I know, counting is the only procedure generally satisfactory, largely because the condition of the organisms (I record "good" and "poor" condition) can be observed in detail, and partly because the relative members of particular species (or other groups), and percentage of debris can be noted.

So far as my experience indicates, dipping (by "closing bottle" below the surface) is the only really efficient way of obtaining the desired quantity of "natural water." It can be used just as well in a shallow pool (by drinking cup, etc.) as in deep water. While No. 25 silk bolting cloth has been standard for catching phytoplankton (especially marine plankton diatoms) for years, it is entirely possible that something else will do as well for filtering, e.g., a smooth filter paper or some cloth other than silk. A little study of results obtainable with different filtering materials may show some other to be as dependable as silk. Any kind of filtering material as well as any kind of container will hold out some of the more adhesive or agglutinative organisms. Even silk is not free from this defect. Absolute accuracy in estimating the density of a population is not attainable in any case. Therefore one should decide what dependability of results he may hope to reach and plan his work accordingly if he desires to undertake the more complicated studies of populations.

In conclusion, perhaps biology teachers interested in research which can be carried along with their teaching may appreciate the results of my own experience. Knowing that I had little time for research and that that little could not be distributed accurately, I selected the field of phytoplankton deliberately. I was reasonably sure of being able to make collections in the river twice a week at one station and once a week at two others. Usually, I could be sure of making a hasty examination of the living material just after catching. If not, I could do so often enough to be able to recognize the different forms in the preserved materials. The detailed studies (mainly enumeration, calculations of numbers, etc.) had to be done at such times as could be found at night, on holidays, etc. Thus the investigation as a whole was about as flexible in requirements as could be expected in any field. However, I must admit that the enormous amount of arithmetical and clerical work might have defeated me if my wife had not come to my rescue.

Contribution No. 189

Reprinted from Analytical Edition
INDUSTRIAL AND ENGINEERING CHEMISTRY
Vol. 15, Page 42, January 15, 1943

Copyright 1943 by the American Chemical Society and reprinted
by permission of the copyright owner

A Versatile Continuous Reading Thermionic Voltmeter

L. J. ANDERSON[1] WITH **J. C. HINDMAN**

Scripps Institution of Oceanography, University of California, La Jolla, Calif.

THERMIONIC instruments for potential measurement can be roughly divided into two classes: vacuum tube voltmeters for voltage measurements in low-resistance circuits and electrometers for voltage measurements in high-resistance circuits. Few attempts have been made to combine into one instrument those characteristics necessary for high-resistance work with desirable features usually found only in vacuum tube voltmeters. The increasing use of the glass electrode and the phototube have put a premium on the development of such universal instruments.

A satisfactory laboratory voltmeter for all-purpose work should be designed with the following characteristics in mind:

Necessary	Desirable	
Low grid current	Continuous reading	Variable sensitivity
Stability	Simplicity of operation	Circuit simplicity
High sensitivity	Wide operating range	Low cost of construction
	Low drift rate	Portability

All these characteristics are not entirely compatible. The great majority of potential measuring circuits having the "necessary" characteristics have been designed around special electrometer tubes of relatively high cost or are circuits suitable only for use as null-point indicators (*4, 6, 8, 11, 13, 14, 15, 18, 22, 28, 29*). None of these instruments has enough of those characteristics listed as desirable to make it suitable for general laboratory use.

For potentiometric titration work an instrument that continuously indicates the potential is most desirable, since it allows the course of the equilibrium to be followed at all times. While several continuous-reading titrimeters have been described (*10, 12, 25, 32, 33*), few continuous-reading voltmeters suitable for high-resistance work are to be found in the literature (*16, 24, 31*). Of the circuits published, that of Penther,

---

[1] Present address, U. S. Navy Radio and Sound Laboratory, San Diego, Calif

Rolfson, and Lykken (*24*) is the most promising for work in high-resistance systems. The principal limitation of their instrument is that it has a range of linear response of only 0.110 volt. Further voltage changes must be compensated for with a step-potentiometer. In titrations of a routine nature where speed is important, it would be more advantageous to have an instrument with a wider voltage range requiring no adjustment during the course of the titration.

The circuit described below was developed in an attempt to combine in one instrument as many of the listed features as possible.

Circuit Synthesis

Recently Garman and Droz (*10*) designed a simple vacuum tube voltmeter employing the bridge-type circuit usually found in stable electrometers. The tube used in their circuit is an ordinary pentagrid converter, type 1A7GT, which has the necessary characteristics for an electrometer tube. The 1A7GT is particularly useful, since it also has a low filament current, with resulting low heat emission and battery drain.

In the circuit of Garman and Droz the plate and anode grid of the pentagrid converter are connected as a conventional Wheatstone bridge. The voltage to be measured is impressed between the control grid and filament and results in a simultaneous increase in plate current and an equal decrease in anode grid current or vice versa. This results in doubly unbalancing the bridge. In their original circuit Garman and Droz connect a sensitive current meter across the bridge to indicate the degree of unbalance. A high-resistance meter would be desirable, to build up a maximal potential difference across the bridge, but a low-resistance meter would give a greater deflection for a given voltage difference.

Experiment showed that, in the voltmeter described, increasing the meter resistance about 90 times decreased the sensitivity only threefold, indicating that the high resistance allowed 30 times as high a voltage to be developed across the bridge as the low resistance. The ideal meter, then, should have a very high resistance as well as a high sensitivity.

The ideal meter characteristics are approached by connecting across the bridge of the Garman-Droz circuit an ordinary vacuum tube voltmeter (Figure 1). The second stage is, in effect, a voltmeter having a resistance of several megohms but with a sensitivity of about 850 microamperes per volt. The 1E4G tube was chosen for this stage because it has advantages over other voltage amplifiers in possessing a low filament current, high transconductance, zero grid bias, and the high stability inherent in triodes.

To this basic circuit were added several other features for increasing the versatility of the instrument. One of these is the potentiometer circuit, R_1-V_1, in the *EMF* input lead to the filament of the electrometer tube.

The bridge resistances, R_2 and R_3, are separate variable resistances to permit varying the instrument sensitivity as desired (see Figure 2). The top view of the instrument (Figure 3) shows the relative positions of R_2 and R_3. For operation at a given maximum sensitivity, either the plate resistance, R_3, or the anode grid resistance, R_2. may be fixed. S_5 and R_8 are included in order that the microammeter may be used for balancing the bridge.

Stadie, O'Brien, and Laug (*30*) point out the desirability of increasing the capacity of the control grid by connecting to it a suitable grounded condenser, C_1. This increased capacity serves

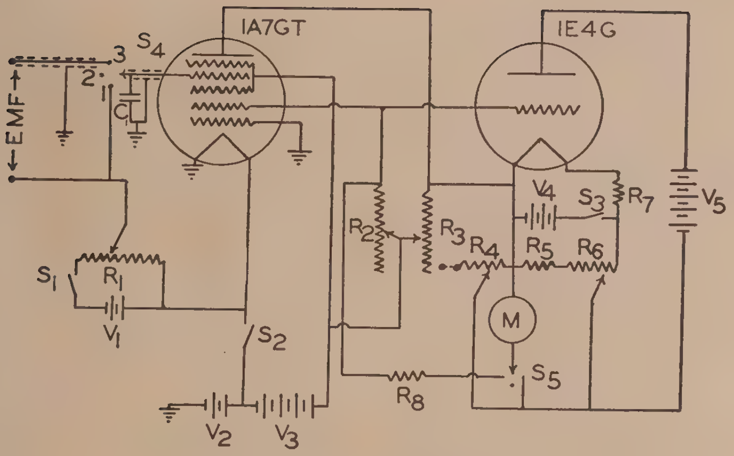


FIGURE 1. VOLTMETER CIRCUIT

C_1.	Tested condenser, capacity 0.02 mfd.
M.	0–500 (120-ohm internal resistance) or 0–100 (900-ohm internal resistance) Triplett microammeter
R_1.	10,000-ohm uniform volume control
R_2.	5000-ohm uniform volume control
R_3.	10,000-ohm uniform volume control
R_4.	2000-ohm uniform volume control (for use with 0–500 microammeter) with switch cover plate for S_3
R_5.	5000-ohm 0.5-watt
R_6.	5000-ohm tapered volume control with switch cover plate for S_1 and S_2
R_7.	30-ohm 0.5-watt
R_8.	2000-ohm 0.5 watt
S_1, S_2.	Double-pole single-throw switch
S_3.	Single-pole single-throw switch
S_4.	Special high-resistance SP3 position switch with off position in center
S_5.	SP3 position switch with off position in center
V_1.	2 1.5-volt flashlight cells
V_2.	1.5-volt filament battery
V_3.	45-volt plate battery
V_4.	2 1.5-volt filament batteries
V_5.	2 45-volt plate batteries

to protect the grid potential from momentary changes due to the opening of switch S_4. In order to prevent leakage currents, this condenser must have a resistance at least as high as the internal resistance of the control grid. To reduce leakage further it is necessary to construct S_4 and the input jack in the grid lead with a high degree of insulation from ground. Many suitable switch designs have been published (*6*, *13*, *28*, *30*). The authors have found that glass tubing makes satisfactory stand-off insulators for this purpose. These can be fastened to the chassis by means of a short bolt and de Khotinsky cement. The jack in the grid circuit was designed to take the Beckman glass electrode fittings. The electrometer tube is shielded by means of a glass cylinder fitted over the tube base and covered with a metal cap connected to ground. The shielded lead to the grid of the electrometer tube passes through this metal cap (Figure 3). The surface of the 1A7GT and the glass cylinder are covered with paraffin to prevent leakage of surface currents. The control grid lead is the only part of the circuit where extraordinary care with regard to insulation need be taken.

In the second stage there are two necessary controls. One of these, R_4, varies the over-all sensitivity by shunting the microammeter. A value of R_4 equal to about 15 times the meter resistance gives a good spread of the sensitivity control with only 6 per cent reduction in maximum sensitivity due to shunting. This

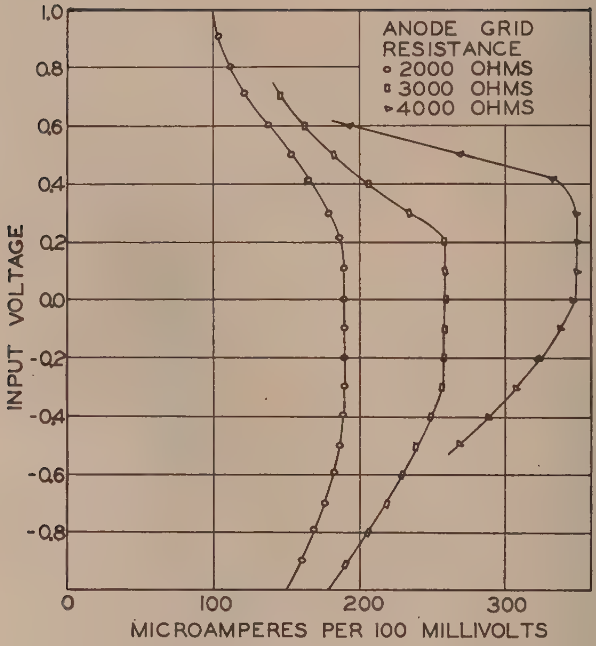


Figure 2. Sensitivity Variation with Bridge Resistance

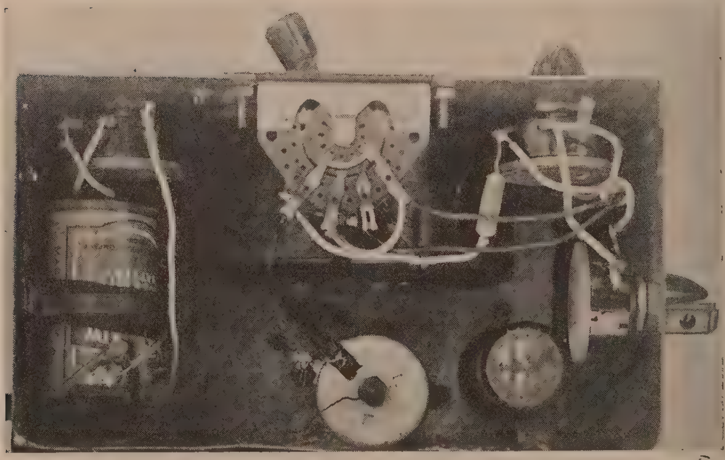


Figure 3. Top View of Voltmeter, Showing Tube Compartment

reduction can also be eliminated if contact is broken at the extreme end of the shunt. Coating the end of the contact surface with an insulating material is a convenient way to do this. The other control, R_6, is in the widely used D-battery circuit (*9*, *26*), which is composed of R_5 and R_6 and the 6-volt filament battery. For a given setting of R_6 a fixed amount of plate current is by-passed around the meter, thus allowing the use of a more sensitive meter. The resistance of both R_5 and R_6 should be kept high to reduce battery drain and to increase the by-passing range. Under these conditions, a small rotation of R_6 causes a large change in the amount of current by-passed. For ease of control a vernier is desirable. An ordinary planetary control with a 5 to 1 ratio has been found satisfactory. The combination of the filament and D-batteries is a commonly used method of stabilizing the circuit against changes in battery voltages (*16*, *19*, *25*, *31*).

The entire instrument with all batteries is enclosed in a 7 × 8 × 10 inch metal cabinet. Figures 3 and 4 show the panel layout and some details of the internal arrangement.

Operation and Adjustment

The preliminary steps in the operation of the instrument are: (1) S_4 is opened (position 2, Figure 1); (2) S_1 and S_2 are closed by rotating R_6 and the rotation is continued to about the center of the range; (3) S_3 is closed by rotating R_4.

In the preliminary adjustment of the instrument it is necessary at this point to balance the bridge by varying R_2 or R_3. At least an hour should be allowed for the electrometer tube to reach equilibrium before this adjustment is made. Switch S_4 is opened and then the microammeter in the second stage is temporarily connected across the bridge by closing S_5. The plate resistance, R_3, is fixed at a convenient value and the anode grid resistance, R_2, is adjusted until the current through the meter is zero. This adjustment need only be checked occasionally after the tube has sufficiently aged. When the adjustment has been made the meter is again switched back into the second stage.

Finally, the free-grid potential is located as follows: (1) With the sensitivity considerably reduced, S_4 is connected to the internal biasing battery (position 1, Figure 1). (2) The bias is adjusted by rotating R_1 until the meter gives the same reading as with the grid floating. The sensitivity is now increased and R_1 further adjusted until no drift or deflection occurs in the meter on opening and closing S_4 to the internal bias. The needle is always kept on the scale by means of R_6.

With these preliminary adjustments completed, the instrument is ready for use. The subsequent procedure depends on the type of measurement to be made.

For potentiometric titrations with low-resistance electrodes the connection of the electrodes is immaterial. The initial electrode potential is set to the free grid with the biasing control. The needle is set either to the upper or lower end of the meter scale with the D-battery control, depending upon the direction of voltage change expected during the titration. The circuit is so connected that the microammeter needle is deflected downscale as the control grid becomes more negative.

When high-resistance electrodes such as the glass electrode are used, they are always connected to the grid lead, since one side of any high-resistance circuit must be highly shielded and common practice is to shield the glass electrode lead. The starting position of the titration will then depend on whether this electrode is to become more positive or more negative. In order to keep the grid current low it is desirable to keep the grid potential within a certain range relative to free grid, determined by the grid current characteristics of the particular tube used. To stay within this range during a titration, the needle is reset with the bias control as it approaches the end of the meter scale. This allows the use of maximum sensitivity, if desired, over a voltage range more than equivalent to 14 pH units.

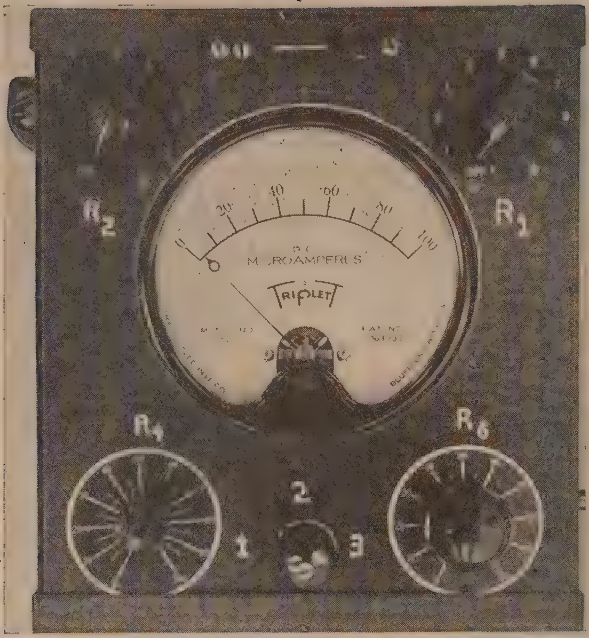


Figure 4. Front View of Voltmeter

If it is desired to use the instrument as a null-point indicator, it is necessary simply to adjust the grid potential with the biasing control to the free grid potential for zero input voltage and to balance out the electrode potential with a precision potentiometer inserted in the low-resistance lead.

Operating Characteristics

The grid current of the electrometer tube must be kept low for two reasons: to ensure an accurate voltage response and to prevent appreciable polarization in the electrode system (*2*). The first of these is important only in measuring circuits employing high-resistance electrodes. For a given accuracy of voltage measurment the grid current becomes the factor which limits the resistance of the electrodes that can be used.

The prevention of polarization is important for all electrode systems. According to Müller (*20*) the limiting current depends upon the type of cell and the time of current flow. In glass electrode systems, Morton (*19*) has said that currents of 10^{-10} to 10^{-9} ampere do not cause appreciable polarization. Robertson (*27*) found in working with electrodes of 2- to 3-megohm resistance that more than 6×10^{-9} ampere was required to give measurable polarization in one minute. As Figure 5 shows, grid currents of 10^{-10} ampere are never reached in the voltage range recommended for the operation of this instrument. As an additional check with the present instrument the following experiment was performed:

With a potentiometer connected in the *EMF* circuit, the potential of a glass electrode (300-megohm resistance)–calomel cell in a buffer solution was determined. The potential was then adjusted to -0.2 volt with respect to free grid. The potential of the cell as indicated by the meter reading showed no change in 30 minutes when corrected for the instrument drift. The electrodes were then disconnected for 5 minutes. When reconnected, they gave the same potential as originally measured by null-point determination. The current flowing through the cell was about 3×10^{-11} ampere. Subsequent work with the same glass electrode has shown that no measurable polarization occurs when titrations requiring several hours for completion are carried out by the recommended procedure.

The advantages of working at the free grid potential have been thoroughly discussed by Nottingham (*21*) and Rasmussen (*26*). According to these authors, the gird current at the free grid is theoretically zero. On the positive side of the free grid, the current increases sharply, owing to an electron flow to the grid. On the negative side of the free grid, the current flow is in the opposite direction. According to Metcalf and Thompson (*17*), the principal causes of this "positive" current are: (1) ionization of residual gas, (2) positive ions emitted by the filament, (3) thermionic grid emission due to heating of the grid by the filament power, (4) photoelectric grid emission due to both visible light from the filament and x-rays produced by the anode current, and (5) insulation leakages.

By the use of a high order of insulation in the grid lead (special input jack and leads, highly insulated switch S_4, tested condenser, paraffined tube surface, and desiccant) leakage currents have been minimized. The presence of one negatively charged grid and two positively charged ones between the filament and control grid will aid in screening the latter against positive ions emitted by the filament. The extremely low filament power of the 1A7G tube (0.08 watt as compared with the 0.2 to 0.5 watt given as average for electrometer tubes, *3*) should make the thermionic and photoelectric grid emissions small for this tube. Attempts to lower the plate and anode grid voltages and thus reduce gas ionization and x-ray emission resulted in drastic loss of sensitivity. Sacrifice of sensitivity was not deemed advisable, since the grid current was already low enough for most measurements.

Figure 5 shows the grid current characteristics for three 1A7GT tubes, all of which were considered satisfactory for use in the voltmeter. Out of a total of eight 1A7GT tubes tested, six Sylvania and two Ken-Rad, only three tubes were found to be suitable for use; two of these were Sylvanias and one was a Ken-Rad. The other tubes in general had a very high $\partial Ig/\partial E$, so that high currents were found very near the free grid potential. Because of this variability in tubes it is necessary to make selection of the tubes with the instrument itself. The disadvantage of having to select the tubes is one commonly encountered in tubes not specially designed for use in electrometers. Satisfactory tubes are selected by measuring the current at the free grid potential and at suitable voltage intervals on either side of the potential by observing the meter deflection with and without a 5000-megohm resistance in the grid circuit. From the voltage sensitivity and the deflection difference the current is computed by Ohm's law.

The grid current characteristics of these tubes appear to be a function of the bridge balance. Tubes which balance at approximately equal plate and anode grid resistances have high grid currents, whereas tubes having a high plate resistance (low plate current) have a much lower grid current. This current would therefore appear to be intimately related to the amount of plate current, a not unexpected result, when we consider the sources of grid currents discussed above. Unfortunately, the conditions for the highest sensitivity correspond to those where the highest grid currents are found. The free grid current for the tubes found to be suitable was less than 3×10^{-13} ampere within the limit to which the free grid potential could be located by the procedure described. With the unsatisfactory tubes it was not possible to approach the free grid potential close enough to secure as low a current. The grid currents found make the instrument unsuitable for use with circuits having a higher resistance than 30 megohms if an accurate voltage response of 1 millivolt is desired over the operating range indicated or with a higher resistance than 300 megohms if an accuracy of 0.1 millivolt is desired for null-point measurement. This would obviate its use in mass spectrometers or phototube outfits where the resistance is 10^9 to 10^{10} ohms. However, excellent glass electrodes ranging in resistance from 2 to 30 megohms may be purchased commercially or constructed by the experimenter (*5*, *27*).

The foregoing remarks apply to the instrument as constructed. Figure 2 shows that increasing the bridge resistance causes a proportional increase in sensitivity and decrease in range of linear response. When used as a null-point indicator or a short-range voltage indicator in connection with a potentiometer this would be no disadvantage.

The choice of meter used with the instrument would depend on the use for which it is intended. For null-point detection a 0–50 microammeter or galvanometer could be used. As a titrimeter a 0–500 microammeter is satisfactory. Both a 0–100 and a 0–500 microammeter have been used in the present instrument with excellent results. Random fluctuations in the meter readings have not been observed. When dry cells are used for the filament supply there is a drift which decreases to less than 1 microampere per minute after the instrument has been turned on for about 2 hours. The authors recommend the use of storage cells for the filament supplies where practicable, as the meter drift is reduced tenfold by this practice. In all the measurements reported in this paper storage batteries were used. Many experimenters have been successful in minimizing drift by various methods of internal compensation (*1*, *3*, *7*, *23*). However, rate of drift of the present instrument remains relatively unchanged in spite of attempts of the authors to make use of such compensation methods.

An attempt also was made to construct a simple alternating current power supply to replace the batteries and thus elim-

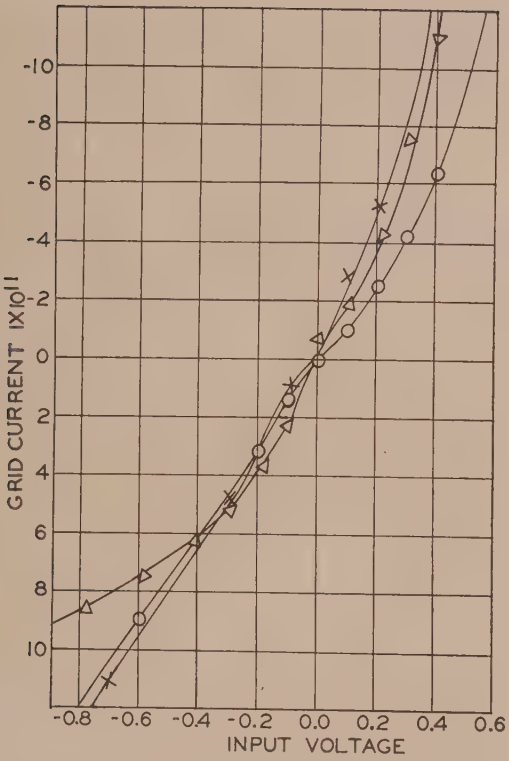


FIGURE 5. VARIATION OF GRID CURRENT WITH INPUT VOLTAGE FOR THREE SATISFACTORY TUBES

inate the drift effects. When voltage regulator tubes of the glow-discharge type were used, it was found, however, that the power delivered could not be controlled within the extremely small limits necessary for complete stability. More complicated regulating circuits, such as those of Working (*34*), would probably be satisfactory if they could deliver sufficient current.

Summary

This article describes a thermionic voltmeter suitable for a wide range of laboratory measurements in both high- and low-resistance circuits.

A description of the circuit, its characteristics, and operation is given and the following uses are indicated: (1) as a direct, continuous-reading titrimeter for glass electrode systems, (2) as a direct continuous-reading titrimeter for low-resistance electrodes, and (3) as a null-point indicator in connection with a potentiometer. The instrument has proved very satisfactory in actual operation over a period of several months in each of these applications. Further applications such as the measurement of photocell currents, etc., in circuits of moderately high resistance are indicated.

The total cost of the materials used in the instrument less batteries was about $25. The particular batteries used cost $5. The low current drain ensures long life for these batteries.

Acknowledgment

The authors wish to express appreciation to E. G. Moberg of the Chemistry Division for suggestions during preparation of the manuscript, and to thank Ben Kievet and the Hygrade Sylvania Corporation for their courtesy in furnishing six life-test tubes for the grid current measurements.

Literature Cited

(1) Barth, G., *Z. Physik.*, 87, 399 (1934).
(2) Cherry, R. H., *Trans. Electrochem. Soc.*, 72, 33 (1937).
(3) Compton, K. G., and Haring, H. E., *Ibid.*, 62, 345 (1932).
(4) DeEds, F., *Science*, 78, 556 (1933).
(5) Dole, M., "The Glass Electrode", Chap. IV, New York, John Wiley & Sons, 1941.
(6) DuBois, D., *J. Biol. Chem.*, 88, 729 (1930).
(7) DuBridge, L. A., and Brown, H., *Rev. Sci. Instruments*, 4, 532 (1933).
(8) Elder, L. W., Jr., *J. Am. Chem. Soc.*, 51, 3266 (1929).
(9) Fosbinder, R. J., *J. Phys. Chem.*, 34, 1294 (1930).
(10) Garman, R. L., and Droz, M. E., IND. ENG. CHEM., ANAL. ED., 11, 398 (1939).
(11) Gilbert, E. C., and Cobb, A., *Ibid.*, 5, 69 (1933).
(12) Goode, K. H., *J. Am. Chem. Soc.*, 47, 2483 (1925).
(13) Goodhue, L. D., *Iowa State Coll. J. Sci.*, 10, 7 (1935).
(14) Goyan, F. M., Barnes, C. L., and Hind, H. W., IND. ENG. CHEM., ANAL. ED., 12, 485 (1940).
(15) Hemingway, A., *Ibid.*, 7, 203 (1935).
(16) Kinney, G. F., and Garman, R. L., *J. Chem. Ed.*, 13, 190 (1936).
(17) Metcalf, G. F., and Thompson, B. J., *Phys. Rev.*, 36, 1489 (1930).
(18) Morton, C., *J. Chem. Soc.*, 1931, 2977.
(19) *Ibid.*, p. 2983.
(20) Müller, Fr., *Trans. Electrochem. Soc.*, 62, 335 (1932).
(21) Nottingham, W. B., *J. Franklin Inst.*, 208, 469 (1929).
(22) Partridge, H. M., *J. Am. Chem. Soc.*, 51, 1 (1929).
(23) Penick, D. B., *Rev. Sci. Instruments*, 6, 115 (1935).
(24) Penther, C. J., Rolfson, F. B., and Lykken, L., IND. ENG. CHEM., ANAL. ED., 13, 831 (1941).
(25) Pope, C. G., and Gowlett, F. W., *J. Sci. Instruments*, 4, 380 (1927).
(26) Rasmussen, E., *Ann. Physik.*, Series 5, 2, 357 (1929).
(27) Robertson, G. R., IND. ENG. CHEM., ANAL. ED., 3, 5 (1931).
(28) Rosebury, F., *Ibid.*, 4, 398 (1932).
(29) Stadie, W. C., *J. Biol. Chem.*, 83, 477 (1929).
(30) Stadie, W. C., O'Brien, H. C., and Laug, W. P., *Ibid.*, 91, 243 (1931).
(31) West, L. J., and Robinson, R. J., IND. ENG. CHEM., ANAL. ED., 12, 476 (1940).
(32) Willard, H. H., and Hager, O. B., Jr., *Ibid.*, 8, 144 (1936).
(33) Working, E. B., *Ibid.*, 10, 397 (1938).
(34) *Ibid.*, 10, 434 (1938).

CONTRIBUTION from the Scripps Institution of Oceanography, New Series, No. 189.

PRINTED IN U. S. A.

Contribution No. 191

Reprinted from Biological Bulletin, Vol. 84, No. 1, 106–114, February, 1943
Printed in U. S. A.

STUDIES ON THE LIFE HISTORY OF THE MARINE ANNELID *NEREIS VEXILLOSA*

MARTIN W. JOHNSON

(Scripps Institution of Oceanography, University of California, La Jolla, and the University of Washington Oceanographic Laboratories, Seattle)

STUDIES ON THE LIFE HISTORY OF THE MARINE ANNELID *NEREIS VEXILLOSA*

MARTIN W. JOHNSON

(*Scripps Institution of Oceanography, University of California, La Jolla, and the University of Washington Oceanographic Laboratories, Seattle*)

Contributions from the Scripps Institution of Oceanography, New Series, No. 191

INTRODUCTION

Nereis vexillosa Grube is the most abundant member of the larger annelids of the Pacific northwest fauna. In Puget Sound it attains a size of about 30 cm. but, as will be explained later, some heteronereid individuals may be only about 6 cm. long.

It is characteristically a cold water form occurring intertidally and in shallow water from eastern Siberia, Alaska and southward along the northwest American coast to Santa Barbara, California. Some reports extend its range as far south as San Diego. In the southern range, however, it may have been confused with *Nereis mediator* Chamberlain (Chamberlain, 1919) or with *Neanthes succinea* (Frey and Leuckart) according to Hartman (personal communication). The apparent absence of the specific egg masses, described below, from the southern range lends support to the belief that *N. vexillosa* may not occur in these waters.

The tiny eggs produced by this animal are about 0.2 mm. in diameter; they are spawned in firm, irregular gelatinoid masses, somewhat translucent, and of blue-green, greenish or brownish tints (Figure 1). These colors are most noticeable in the freshly laid eggs owing to their greater compactness prior to absorption of water by the capsular material. The firmness of the masses enables them to withstand a good deal of handling or washing about on the beach by waves without disintegrating. Hence they are often found in good condition on tidal flats where bits of seaweed or other debris collects at the waters edge. They appear never to be found in more than moderate quantities despite the abundance of the species producing them.

In so far as known, there is no other nereid worm that deposits its eggs in this manner. Commonly the eggs of other species of the genus, or of closely related genera are deposited separately in the water or are only lightly agglutinated. The size of masses varies considerably being about one to three inches in diameter, apparently depending upon the size of the female depositing them.

The identity of the egg masses has long been a puzzle to biologists and others alike, and, indeed, the present study was initiated in 1927 at Friday Harbor, Washington, primarily for the purpose of identification. In the course of the investigation, however, important aspects pertaining to the life history of the species have come to light.

It is a pleasure to acknowledge the generous support given this investigation by Director T. G. Thompson, Professor T. Kincaid, and other members of the staff of the University of Washington Oceanographic Laboratories where facilities

were provided for much of the work. My thanks are also due to Dr. Olga Hartman of the Allen Hancock Foundation for identification of the heteronereis worm and for helpful suggestions.

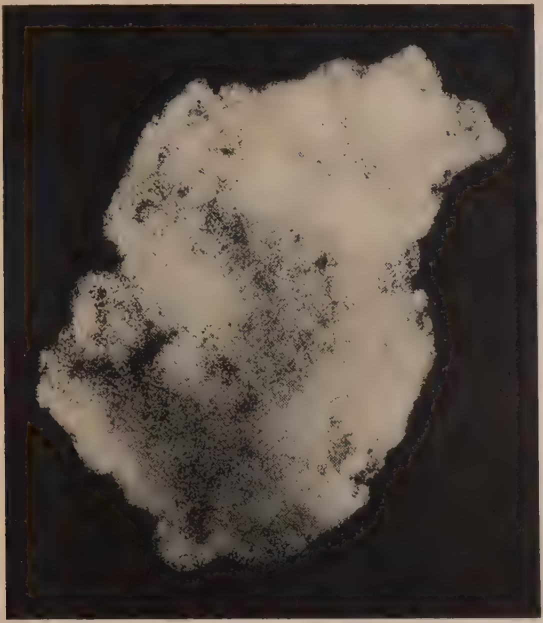


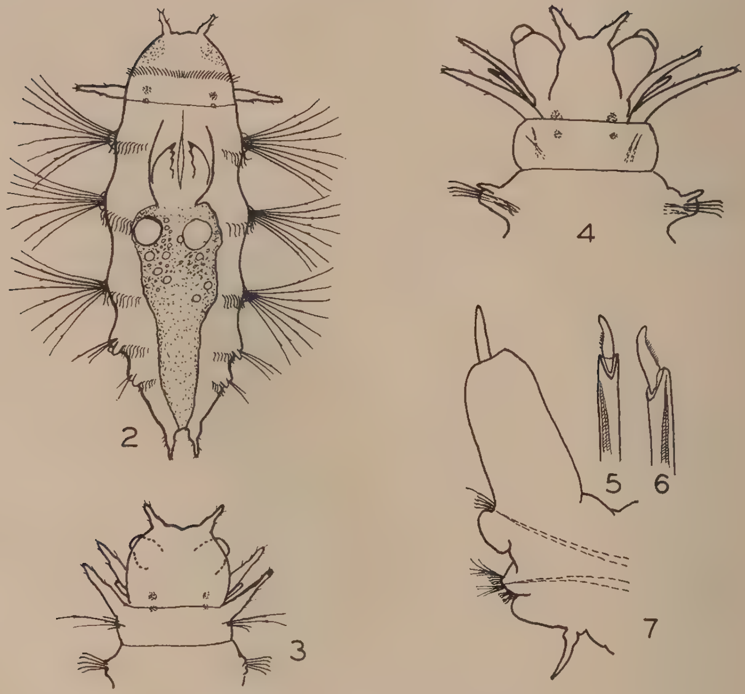
FIGURE 1. *Nereis vexillosa.* A small egg mass, about natural size.

CULTURE EXPERIMENTS AND DEVELOPMENT

The larvae resulting from culture of eggs collected in 1927 failed to survive long beyond hatching and therefore served to establish only that they were annelids of the nereid type. The failure of these to survive may be due in part to inadequate food since they were fed only diatoms and algae. This diet brought good results with *Platynereis agasszi* which was being reared at the same time (see Guberlet, 1933), but subsequent experiments have indicated that *Nereis vexillosa* thrives best in later life when animal food is provided.

In the summer of 1941 an opportunity was again presented to set up cultures at Friday Harbor. Two egg masses were found in False Bay and rearing of the larvae began on June 28. Some of the larvae were already hatching in the three to five segmented stage (Figure 2). The larvae and young worms grew slowly and were able to survive for about a week after hatching on the yolk content which became concentrated in the digestive tract. Later they were fed sessile diatoms (*Navicula sp.* and *Biddulphia laevis*) and powdered dry scallop muscle.

The rate of growth varied so greatly that on August 23 the most advanced worms, now about 57 days old, had acquired 35 segments and were about 9 mm. long, whereas the least advanced had but 15 segments and were about 5 mm. long.



FIGURES 2–7. *Nereis vexillosa.*

FIGURE 2. Five-segmented larva.

FIGURE 3. Head of eight-segmented larva to show development of peristomial tentacles.

FIGURE 4. Head of fifteen-segmented larva to show further progress of head development.

FIGURES 5 and 6. Homogomph notoseta and heterogomph neuroseta from parapodium shown in Figure 7.

FIGURE 7. Parapodium from posterior portion of body of eleven month old worm.

(Camera lucida drawings, Figures 2, 3, 4 same scale; Figures 5, 6, 7 enlarged scale.)

It was, however, still not possible to establish identity of the worms from the most advanced specimens, therefore, at the end of the summer session the animals were moved alive to the Scripps Institution of Oceanography at La Jolla, California. This was accomplished by placing ten specimens of various sizes in each of four vials with sea water and containing a wad of cotton among the strands of which the young worms found protection against violent battering in the vials while in transit. The vials were kept cool by being wrapped in wet, frequently-changed paper towels.

Upon arrival at La Jolla the specimens were changed gradually to local sea water (salinity about $3^{0}/_{00}$ above that at Friday Harbor) in small culture dishes and it was found that 25 had survived the journey. Following some additional mortality in the first month, 16 of various sizes lived for several months.

After setting up the cultures at La Jolla, about three-fourths of the water was changed in each dish every two or three days and the animals were fed dried powdered pecten and sea mussel for a few weeks, but growth was so slow that it was decided to try feeding finely chopped pieces of freshly killed *Thoracophelia mucronata*, a polychaete worm found abundantly at La Jolla. This fresh food was given in small quantities every second or third day. With it there was a prompt response by more rapid growth. Later bits of fresh mussel were also fed but the minced worms seemed to be preferred. Not only the killed and minced worms were eaten but living specimens half the size of the young Nereis were eaten. This rapacious habit was evident even in worms only 6 or 8 mm. long that were seen to grasp and completely swallow in a few seconds others of their kind that were fully half their own size. Diatoms were also added to the dishes and these were readily eaten. The animals rejected partially decomposed food and since the object of the experiment was to keep them growing as long as possible no further experimenting with food was deemed advisable. Results indicate that *N. vexillosa* is more or less omnivorous, utilizing mostly animal food but that it is not a scavenger by preference. According to Copeland and Wieman (1924) *Nereis virens* is also omnivorous though Gross (1921) found only evidence of plant feeding.

No membranous tubes were constructed as in *Platynereis agasszi*, only flimsy tubes of sand and debris were formed with the aid of secreted mucus, and after the animals had reached a length of about 15 mm. they rarely left their tubes completely to gather food. To facilitate study, pieces of glass tubing were provided and these were readily accepted in most cases. Some animals refused to accept new glass tubes that were provided to replace those outgrown and deserted. In these instances the lack of security resulted in restlessness and failure to feed normally.

The older specimens were kept in running (uncooled) sea water pumped in from the sea. At the middle of July this water had reached a temperature of 20° C., which is about 6 to 8 degrees higher than might be expected in the natural habits of *N. vexillosa* near Friday Harbor during the summer. This may well have been a contributing factor in the failure of the worms to survive with the advance of summer at La Jolla.

In Table I is given a summary of the condition of the worms that survived ten or more months. Two of these were found dead (May 18 and May 29). The others were killed and preserved only after they had deserted their tubes and it seemed obvious that they would not survive much longer.

At the age of four and one-half months, when about 60 segments had been formed, the worms reared from the 1941 egg masses had developed specific characteristics of *N. vexillosa*. Especially characteristic are the elongate strap-like dorsal ligules (Figure 7) of the parapodia of the posterior region of the body, but the head structures and the setae of the posterior region are also distinctive (Figures 5 and 6) in older specimens.

The process of cephalization is shown in Figures 2, 3, and 4. In this development, the first setigerous segment of the early larva becomes modified to form the peristomium. The anterior dorsal pair of peristomial tentacles appear first, followed by the posterior dorsal pair which develop from the first larval parapodium. A ventral pair of anterior peristomial tentacles then appear and finally the posterior ventral pair of tentacles are in evidence when the worm has acquired about 18 to 20 segments.

TABLE I

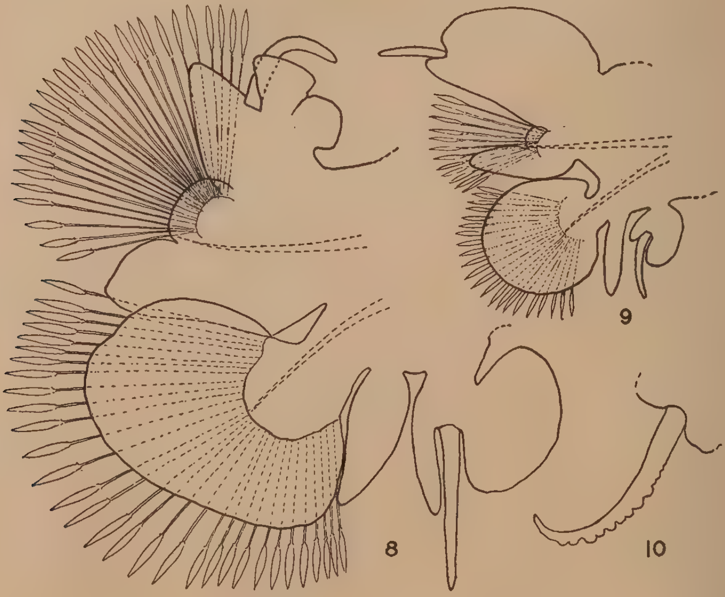
Nereis vexillosa. The seven oldest specimens from cultures set up at Friday Harbor, June 28, 1941

Date killed or found dead 1942	Number of segments	Approximate length when moderately relaxed
April 25	128	8 cm.
May 18	109	5 cm.
May 26	109	6.7 cm.
May 26	107	4.5 cm.
May 29	119	8.5 cm.
June 10	105	7 cm.
July 28	125	10 cm.

None of the specimens showed any indication of entering the heteronereis phase. In this respect they differ from *Platynereis agasszi*, several of which were found to enter this phase near the end of the first year.

SPAWNING HABITS

While culturing the larvae at Friday Harbor in 1941 a watch was kept for spawning adults in the bay. Finally from isolated small, spawning heternereids there was obtained several masses of eggs. These spawners proved to be *Nereis*



FIGURES 8–10. *Nereis vexillosa.*

FIGURE 8. Heteronereized female parapodium, middle portion of posterior body.
FIGURE 9. Heteronereized female parapodium, fourth from last segment.
FIGURE 10. Dorsal cirrus, heteronereized male parapodium.
(Figures 8, 9, 10 camera lucida drawings to same scale.)

vexillosa Grube thus confirming the identification of the worms reared experimentally from egg masses collected in the field. Heteronereized parapodia are shown in Figures 8 and 9. Heteronereis males have 25 parapodial segments in the anterior portion of the body while the females have 27 such segments (count of 12 males and four females). In the males the dorsal cirrus of the heteronereized segments bears a series of wart-like protuberances on the ventral surface (Figure 10) while in the females examined these cirri were smooth.

The spawning worms were obtained only at night while collecting with a light at the end of the pier in front of the laboratories. They appeared only in small numbers usually an hour or two before midnight and were mingled with spawning swarms of the smaller species *Platynereis* (formerly *Nereis*) *agasszi* which on all occasions was the first of the two to appear swimming at the surface. It was not possible with these few observations to establish any correlation of spawning with phases of the tide or moon as has been done with other marine worms (cf. Woodworth, 1907; Lillie and Just, 1913; Guberlet, 1933).

Only small individuals of *N. vexillosa* were seen spawning and from what has subsequently been learned through the above rearing experiments, it seems certain that these were all spawning for the first time at the age of one year. Not only were some of the experimentally reared worms as large at the age of 10 to 11 months as some of the spawning worms but there was also indication of approach to sexual maturity. One of the worms reared at La Jolla was killed April 25, 1942 after having deserted its tube. Upon examination it was found to contain many eggs which were very small and not yet ripe, but their large numbers might be interpreted to indicate that spawning would normally have occurred in the coming summer. A second worm that died May 29 also showed many eggs. However, it seems certain that in nature some heteronereis individuals must be older than one year at spawning since egg masses of much greater size than those known to have been spawned by the smaller specimens have been found. This is more fully discussed later.

In nature more males than females were observed swimming at the surface. They are the first to come to the nuptial party where, as scattered individuals, they suddenly appear from below and rise to the immediate surface, swimming a few moments there in spirals and loops and then disappearing into the deeper water or the darkness beyond the range of the collector's light. They continue unabated in numbers and vigor as the females a little later appear to join the dance, a dance which seemingly is a climax that marks the ends of their lives, for none of the exhausted individuals kept in captivity was observed to live more than a few days following the act of spawning.

Isolated small heteronereis females ripe with eggs were induced to spawn almost instantly when a few drops of sperm laden water were added to the water in which they were isolated. This is similar to the findings of Lillie and Just (1913) for *Nereis limbata* and of Just (1929) for *Platynereis dumerilii*. In large battery jars the act of spawning by the female *Nereis vexillosa* consists of coming to or near the surface and suddenly exuding a mass of eggs which instantly agglutinates (Figure 11). She then passively sinks to the bottom together with the mass. A few moments later she frees herself from the mass which remains demersal and which in a few hours swells to about three or four times its original size through the absorption of water. The spawning of these demersal masses in water just beyond the low tide limit may account for the finding of fewer egg masses than are commensurate with the number of worms.

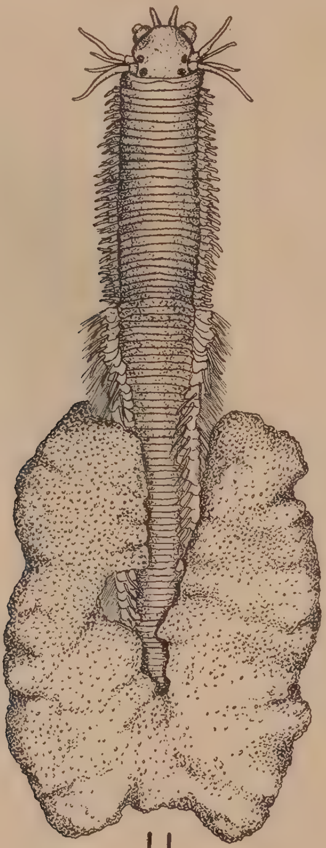


FIGURE 11. *Nereis vexillosa.* Method of egg deposition by small heteronereis. (Free hand drawing.)

DISCUSSION

An answer has been found as to the identity of the egg masses, and certain features of the life history of the species have been discerned. In the process of interpreting the observations, however, another biologically important question arises, namely, does *Nereis vexillosa* possess a diversified life history in which there may be recognized several types of reproductive individuals, as indicated for *Platynereis dumerilii* by Hempelmann? In the latter species at Naples, Hempelmann (1911) distinguished (1) small sexually mature nereis (i.e., atokus) individuals that gave rise to heavily yolked eggs producing characteristic larvae which he called "nereidogene"; (2) small heteronereis forms producing less heavily yolked eggs developing into pelagic larvae called "planktogene"; (3) large heteronereis forms with eggs as in the small form but whose larvae have not been investigated. He found that after spawning, the small nereis form may in experimental cultures be transformed into a small heteronereis form and produce young for the second time and is therefore dissogenous. The nereis form may also grow to a relatively large size and then transform to the large heteronereis but the steps involved in arrival at the large form in this phase are uncertain. It is believed that entrance into the heteronereis form and its spawning marks the end of life for the individual.

More recently Just (1929) also worked on the Naples species and in so far as his investigation was carried, the findings of Hempelmann were verified.

It has been noted by H. P. Johnson (1901) that the heteronereis form of *Nereis vexillosa* occurs in individuals of 56 mm. and upward in length but the maximum length is not given. The same author reports that sexual maturity is frequently arrived at by the species without it becoming heteronereized. Ricketts and Calvin (1939) report finding many large heteronereis of *N. vexillosa* but the authors never found these to be free-swimming. The supposition is, however, that they do spawn, and indeed the finding of egg masses in Puget Sound that are much larger than those known to have been spawned by small heteronereis forms of the species substantiates this.

What the destiny of the worms reared from the Friday Harbor material would have been normally can be only a matter of conjecture. In this connection it may be significant to note that the number of segments in several large (14 to 21 cm. long) *N. vexillosa* collected in the field at Friday Harbor was 142 to 152 in the nereis phase, whereas the spawning heteronereids taken in the same region had only 63 to 96 segments. The latter figure is less than the number occurring in the seven most advanced specimens reared in cultures (Table I). These specimens had 107 to 128 segments and the length of some was greater than the spawning worms. This may mean that the reared worms were destined to reach sexual maturity only in a more advanced nereis phase or in a large heteronereis phase. Much additional study is needed to answer this question. The great range in size that is possible in the heteronereis of *N. vexillosa* should make it an ideal species for such a study if culture problems can be overcome.

SUMMARY

Nereis vexillosa deposits its eggs in firm irregular gelatinoid masses which vary in size from about one to three inches in diameter.

Spawning of small (6 to 8 cm. long) heteronereids of this species was observed to take place an hour or two before midnight. The eggs which are demersal are apparently spawned in water at or just beyond the extreme low tide level and this habit may account for the finding of fewer egg masses than seems commensurate with the number of the species producing them. Isolated heteronereis females were induced to discharge their eggs by the introduction of spermatozoa into the water.

Worms cultured in the laboratory from egg masses collected on the beach throve best on fresh animal food. A number of young worms transported from Friday Harbor to La Jolla attained a maximum length of 10 cm. at the age of 13 months after hatching. All had acquired specific characteristics but none became heteronereized. The transformations taking place during cephalization are similar to that occurring in other nereid worms.

Since the above maximum size of the reared worms is comparable to that of the small spawning heteronereis forms, it appears that the latter were spawning at the age of about one year. Much larger nereis and also heteronereis individuals are known to occur in this species but the time and steps involved in their development are unknown.

LITERATURE CITED

Chamberlain, R. V., 1919. New polychaetous annelids from Laguna Beach. *Pomona Jour. Entom. and Zool.*, **11**: 1–23.
Copeland, M. and H. L. Wieman, 1924. The chemical sense and feeding behavior of Nereis virens Sars. *Biol. Bull.*, **47**: 231–238.
Gross, A. O., 1921. The feeding habits and chemical sense of Nereis virens Sars. *Jour. Exp. Zool.*, **32**: 427–442.
Guberlet, J. E., 1933. Observations on the spawning and development of some Pacific annelids. *Proc. Fifth Pac. Sci. Congr.*, **5**: 4213–4220.
Hempelmann, P. F., 1911. Zur Naturgeschichte von Nereis dumerilii Aud. et Edw. *Zoologica*, H. 62, 1–135.
Johnson, H. P., 1901. The Polychaeta of the Puget Sound region. *Proc. Boston Soc. Nat. Hist.*, **29**: 381–437.
Just, E. E., 1929. Breeding habits of Nereis dumerilii at Naples. *Biol. Bull.*, **57**: 307–310.
Lillie, F. R. and E. E. Just, 1913. Breeding habits of the heteronereis form of Nereis limbata at Woods Hole, Mass. *Biol. Bull.*, **24**: 147–160.
Ricketts, E. F. and J. Calvin, 1939. Between Pacific Tides. 320 pp., Stanford Univ. Press, 1939.
Woodworth, W. McM., 1907. The Palolo worm, Eunice viridis (Gray). *Bull. Harvard Mus. Comp. Zool.*, **51**: 3–21.

Contribution No. 192

Influence of Bacterial Activity On Source Sediment

Research studies show bacteria convert organic matter into substances which are more petroleum-like. Thus they may play an important role in the formation and accumulation of oil. Further study may reveal that bacteria provide clues to detection of present subterranean deposits

By CLAUDE E. ZoBELL
Scripps Institution of Oceanography, University of California, La Jolla

Reprinted from THE OIL WEEKLY, April 26, 1943

BACTERIA and allied microorganisms may play an important role in the formation and accumulation of petroleum. They may even provide a clue to the detection of present subterranean deposits.

Although there is no experimental evidence to indicate that bacteria can convert organic matter into crude oil, bacterial activity may promote the process in various ways. Likewise the activities of other kinds of bacteria may prevent the accumulation of petroleum hydrocarbons in certain environments.

What Are Bacteria

Bacteria are simple single-celled organisms having very poorly differentiated protoplasm. It is still indeterminate whether they possess a definite nucleus. With very few exceptions it is impossible to recognize species, or even genera, by their morphological appearance, although their size, shape, cell arrangement, flagella, capsules, granules, endospores, and other structures may be helpful in characterizing bacteria.

Ideally there are three principal morphological types of bacteria: spheres which are called *cocci*, rods which are called *bacilli* and curved rods which are called *vibrio* or *spirilla*. However, most of those occurring in recent sediments are highly pleomorphic, incongruous, bizarre forms which defy description in simple geometric terms. Progeny of the same species may be morphologically as unlike as particles of clay are unlike each other.

They are very small, being only about one micron (1/25,000 inch) or less in diameter. Since bacteria must be differentially stained and magnified about a thousand times to be seen, they escape detection by the microscopes ordinarily used by the petrologist or paleontologist. In fact, it is very difficult, if not impossible, to recognize bacteria in sedimentary materials, particularly when the latter are rich in organic debris.

Biochemical Activities of Bacteria

Bacteria multiply by transverse fission. Under favorable conditions they may divide every 20 or 30 minutes. At this rate of multiplication the progeny of a single spherical cell, one micron in diameter, would make a mass the size of the earth in three or four days if all survived.

The growth or multiplication of bacteria is limited by a lack of nutrients, unfavorable environmental conditions, natural enemies and by the toxic end-products of bacterial metabolism. Under favorable conditions the ability of bacteria to multiply and to assimilate organic matter is almost unbelievable. About one third of the organic matter which they assimilate is converted into bacterial protoplasm and the rest is oxidized to carbon dioxide and water or intermediate products.

There is virtually no kind of organic matter which is not susceptible to attack by some kind of bacteria, and the intermediate products of metabolism are countless. Bacteria play an important role in soil fertility,[1] and scores of bacterial species are used in industrial processes[2] which require the rapid transformation of organic matter. The importance of bacteria in the carbon, nitrogen, sulfur and phosphorous cycles in nature is well known.

As will be elaborated below, the biochemical activities of bacteria are not restricted to the transformation of organic matter. They have a direct effect upon many minerals and an indirect effect upon more.

In order to demonstrate the presence of bacteria in sedimentary materials, the bacteriologist cultivates them in enrichment cultures until they can be recognized by their mass or biochemical products. The commonest method is to inoculate nutrient media with a known small quantity of the material suspected to contain bacteria. Bacteria may continue to multiply in a clear liquid medium until the latter is cloudy or turbid, or the multiplying bacteria might induce changes in the pH or other chemical properties of the nutrient medium which can be detected.

Sometimes a solid medium in covered dishes (Petri plates) will be inoculated with bacteria. If there are only a few bacteria in the inoculum and they are evenly distributed in the nutrient medium, they may develop into discrete colonies which are a millimeter or so in diameter. The number of colonies which develop is indicative of the number of bacteria present in the inoculum.

It should be obvious that such methods detect only a small percentage of the living bacteria. Several cells sticking together or adhering to a tiny particle will give rise to only one colony and will be counted as only one. Furthermore, the nutrients in the medium, its pH, osmotic pressure, surface tension or oxygen content may be suitable for the multiplication of only a few types of bacteria present. Also the temperature at which the medium is incubated influences the growth of bacteria. Some like it hot and some like it cold.

At their best, nutrient media detect no more than 1 to 10 percent of the bacteria with which we are familiar. There is no basis for estimating what percentage of the bacteria present in new or unexplored horizons will be dedetected by the conventional procedures, but judging from experiences with soil, water or intestinal bacteria, it can be tacitly assumed that the percentage is very low.

A few years ago reports were current that there were few or no living bacteria on the sea bottom. This conclusion resulted from the failure of investigators to find bacteria from the sea bottom developing in nutrient media which were designed primarily for the growth of soil or fresh-water forms. As new media have been perfected to satisfy the cultural requirements of marine bacteria, more and more bacteria have been found in the sea.

If living bacteria are present in ancient sediments or source beds, their cultural requirements may be as different from those found in recent sediments as marine bacteria are different from fresh-water or soil forms.[3]

Occurrence of Bacteria in Recent Sediments

Although only a very small fraction of the area of the sea floor is represented by the few hundred samples which have been bacteriologically analyzed, it is significant that modern methods of analysis have demonstrated an abundant bacterial flora in all of them.

Thousands to millions of living bacteria per gram of sediment have been recovered from different parts of the ocean floor.[4]

Bacteria have been found in sediments several hundred miles from land and in water depths exceeding three miles. Neither water depth nor distance from land seem to influence the abundance of bacteria in marine-bottom deposits except in so far as these factors influence topographical conditions, which in turn might influence conditions of sedimentation or oceanic circulation.

In general, sediments of small particle size will contain larger bacterial populations than those of larger particle size. A high content of organic matter favors large bacterial populations.

The bacterial population decreases rapidly with core depth.[5] The decrease is an exponential function throughout the topmost 5 to 15 inches, depending upon the rate of sedimentation. Below this depth the decrease is slow and sporadic.

Viable bacteria have been recovered from the bottom of the longest cores examined. The analyses include more than a score of cores exceeding 10 feet in length. The longest core examined was about 17 feet long and represented a penetration of around 25 feet. There were more than 39,000 living bacteria per gram in the lower layer of this particular core. Cores from greater depths must be examined in order to define the limits of bacterial activity in sedimentary materials.

Evidence of Bacterial Activity in Sediments

Merely finding living bacteria present in sedimentary materials fails to prove that they are active *in situ* although this observation constitutes pretty good evidence, particularly since most of the bacteria are not spore formers. It is difficult to believe that the bacteria have been buried in a state of suspended animation for thousands or hundreds of thousands of years.

More convincing evidence of their activity in the sediments is provided by chemical analyses. There is a gradual decrease with depth in the organic carbon and nitrogen content of the cores which is attributable to bacterial activity. Moreover, a relationship has been found between the numbers and kinds of bacteria present and the sulfate content of the sediment aswell as the hydrogen-ion concentration at different core depths.[39]

Theoretically there is no known reason why bacteria could not be physiologically active in recent marine sediments. Nutrient material for food and energy is available as indicated by the fact that when samples of sedimentary material are observed in the laboratory, the bacterial population may increase a ten- to a hundred-fold accompanied by the evolution of carbon dioxide and other chemical changes. The increased bacterial activity which occurs when such samples are stored in the laboratory is partly due to the more favorable temperature and partly due to the oxygenation of the material. Other factors are also involved.

Bacterial Enzymes or Biocatalysts

The role of biocatalysts or enzymes must not be overlooked. Most bacteria produce an abundance of a variety of enzymes, the tools with which bacteria work. The enzymes may continue to be active long after the organisms themselves have ceased to exist. The protective effect of adsorbents such as clay particles together with that of the anaerobic conditions which prevail in recent sediments on the sea floor would tend to preserve the enzymes. Such enzymes might activate or catalyze chemical transformations which otherwise would not occur at ordinary temperatures and pressures.

Organic catalysts of various kinds have been found concentrated in recent marine sediments, including bacterial enzymes which cause the oxidation of organic matter in the absence of bacteria. When buried in sediments, such enzymes might have an almost infinite time to function.

Attention should be devoted to learning more about the quantity, distribution, and properties of enzymes that might influence the conversion of organic matter into petroleum.

Effect of Temperature on the Activities of Bacteria

Most marine bacteria are more active at 60° to 70° F. than at the lower temperatures which prevail at the bottom of the sea. (More than 80 percent of the sea floor is perpetually colder than 40° F., and in places it is colder than 32° F.[8]). Laboratory experiments[6] show that many bacteria multiply and are otherwise physiologically active at temperatures lower than any which occur in the ocean. The rate of activity may be slow at low temperatures, but it will be stopped only when the last trace of water has been frozen solid. Sea water of average salinity starts to freeze at around 28.6° F., and brines freeze at much lower temperatures.

In considering the possible importance of bacteria as geological agents, it is not to be surmised from the preceding discussion that bacteria grow only at low temperatures. Some species have an astonishingly high temperature tolerance. Although a good many bacterial species are killed in 10 minutes by exposure to a temperature as high as 145° F. (the pasteurization temperature of milk), bacteria which survive higher temperatures are quite common. Most spore-formers are not killed at 145° F., and some bacterial species grow at this temperature. Thermophilic or thermogenic organisms have been responsible for the spontaneous combustion of manure piles, haystacks and piles of moist rags. Living microorganisms have been found growing in hot springs at a temperature of 192° F. It remains to be seen if there are organisms which live at higher temperatures.

The bacteria which have been studied respond to temperature according to the van't Hoff rule: their biochemical activities are increased a two- to threefold for each 10° C. increase in temperature within their range of tolerance.

Effect of Hydrostatic Pressure

It has been argued that the high hydrostatic pressure which prevails at great depths precludes the possibility of bacteria living there. However, living organisms have been recovered from ocean depths exceeding three miles where the hydrostatic pressure exceeds 500 atmospheres or 7500 pounds per square inch. None of the bacteria studied by Larsen and co-workers[7] were injured by pressures of 3000 atmospheres, and many tolerated pressures of 6000 to 12,000 atmospheres.

A high pressure of carbon dioxide or other biologically active gases may be harmful to bacteria, or sudden changes of pressure may be lethal, but it is generally believed that hydrostatic pressure is not a factor which limits bacterial activity in nature. The hydrostatic pressure of the deepest known depression in the sea,[8] the Minandao Deep which is 35,400 feet below sea level, is only 1100 atmospheres. At depths in the earth's crust where such pressures exist, the temperature is probably too high to be compatible with life of any kind.

Effect of Osmotic Pressure

Marked differences exist in the salt tolerance or osmotic pressure requirements of bacteria. Only a small percentage of the bacterial flora of soil or fresh-water environments can live in sea water.[3] (The average salt content of sea water is 3.4 percent, or 34,000 parts per million, a large proportion of which is sodium chloride.[8]) Doubling the salt content of sea water by evaporation discourages the growth of many marine bacteria but there are some which thrive in concentrated salt solutions.

The high salinity or osmotic pressure of brines associated with petroleum deposits will not prevent the growth of halophilic (salt-loving) bacteria. Such organisms abound and are physiologically active in brines.[9] They occur in the Dead Sea[10] and in Great Salt Lake.[11] The salinity of these bodies of water ranges from 25 to 30 percent. Halophilic bacteria are also found in marine salterns from which salt is crystallizing.

Provided organic matter and moisture are present, no brine is too concentrated for the growth and biochemical activity of halophilic bacteria. There are many kinds of halophilic bacteria which are physiologically versatile.

Kinds of Bacteria

While it is necessary for the bacteriologist to know the generic and species names of bacteria, for present purposes it is preferable to characterize them according to their physiological activities. Facultative aerobes, or those which are active either in the presence or in the absence of free oxygen, predominate in marine-bottom deposits. A few are strict anaerobes which can live only in the absence of free oxygen.

Most marine bacteria are proteolytic, being able to decompose purified as well as natural plant or animal proteins. A good many are saccharolytic, attacking sugars, starches and even cellulose. Some lipolytic bacteria oxidize fats to carbon dioxide and water, but most of them merely hydrolyze the fats and vegetable oils. The resulting glycerol is often oxidized as a source of energy while the fatty acids accumulate. An anaerobic or reducing environment favors the accumulation of the fatty acids liberated by the bacterial hydrolysis of fats and oils.

As stated above, there are bacterial species which can attack nearly any kind of organic matter, including such substances as chitin, lignin, hemicellulose and humus. Of course, there are very few bacterial species which will attack such complex substances and the rate of attack is very slow.

Marine sediments generally contain an appreciable number of bacteria which can reduce nitrates or sulfates. Others activate the transformation of several inorganic or mineral constituents of sediments as noted below.

Effect of Bacteria on the pH of Sediments

Bacteria are probably the principal dynamic agents which are responsible for changes in the hydrogen-ion concentration, or pH, of sedimentary materials.[39] Depending upon the numbers and kinds of bacteria present, the substrate and other environmental factors, bacteria cause the pH to increase or to decrease.

The pH is decreased, or the environment is made more acidic, by bacteria which produce carbon dioxide, oxide organic matter to organic acids, produce nitrites or nitrates, oxidize hydrogen sulfide or sulfur to sulfate or which utilize ammonia. As an extreme example of the ability of certain bacteria to lower the pH may be mentioned a well-known soil organism, *Thiobacillus thiooxidans,* which may continue to oxidize sulfur until the pH falls to less than 1.0. It may produce the equivalent of N/10 sulfuric acid.

Although the pH of sediments may not be greatly reduced, the acid produced by bacterial activity may dissolve calcium carbonate, thereby rendering limestone more porous[12] and retarding the lithification of unconsolidated sedimentary materials. The acid may alter the state of other mineral constituents as well as influencing chemical reactions of various kinds.

Other bacteria increase the pH by the liberation of ammonia from nitrogenous compounds, by the utilization of carbon dioxide or organic acids or by the reduction of sulfates. These bacteria favor the formation of limestone and the cementing action of calcium carbonate.

A high pH favors the preservation of calcareous fossils, a low pH tends to destroy them. The pH also influences the oxidation-reduction potential.

Effect on the Oxidation-Reduction Potential

Bacteria tend to create reducing conditions, or to lower the oxidation-reduction (redox, for short) potential[13] of marine sediments. Whereas sea water itself may be slightly oxidizing in character,[14] bottom deposits may be as reducing as Eh —0.2 to —0.5 volts. The latter is more reducing than the theoretical hydrogen electrode. Such reducing conditions favor the hydrogenation or reduction of organic matter and the preservation of reduced organic matter such as petroleum hydrocarbons, for example.

As a rule marine sediments become more reducing with core depth.[15] Little is known concerning the redox potential of producing horizons although petroleum itself is quite reducing in character. Recently we have found samples of oil shale, prepared for us under the supervision of Dr. K. C. Heald of Gulf Oil Corporation, to be very reducing, although it was exposed to air during the preparation of the samples.

The redox potential of sediments has a pronounced effect upon the direction, velocity and magnitude of chemical reactions which involve the exchange of electrons, or the oxidation or reduction of reactants. Whether iron occurs in the ferrous or ferric state and whether manganese occurs in the manganous or manganic form is largely a function of the redox potential of the environment. The form of other minerals as well as that of organic matter is influenced by the redox potential.[16]

Both the reducing *intensity* and the reducing *capacity* must be taken into consideration.

Effect of Bacteria on Gas Tension

Most bacteria consume oxygen. Consequently they will tend to deplete the free oxygen from a system in which the oxygen supply cannot be replenished from the atmosphere or by photosynthetic activity. This is what happens in marine sediments. Most marine muds contain no dissolved oxygen and they usually have an oxygen deficit. This is shown by the fact that upon mixing with oxygenated water, large quantities of oxygen are instantaneously taken up by the mud.[15] The same is true of the oil shales mentioned above.

Carbon dioxide is produced by most bacteria which oxidize organic matter, and it is utilized by a few. Similarly carbon monoxide is produced by some bacteria and consumed by others. The carbon dioxide and carbon monoxide which occur in marsh gas, and maybe other kinds of natural gas, are quite likely bacterial products.

Denitrifying bacteria liberate free nitrogen from nitrates and nitrites. This reaction appears to be quickly completed in recent sediments because neither nitrates nor nitrites are found, although both may be present in the overlying water.

Methane is produced from most kinds of organic matter by certain bacteria. There is voluminous literature on the factors which influence the bacterial production of methane.[17,18] Maxon[19] has attributed the gas pits in sediments to the production of methane from organic matter by bacteria.

Bacterial Production of Hydrogen

Certain theories of petroleum formation require the presence of hydrogen.[27a] Whether bacteria produce it where needed for petroleum formation is still indeterminate, but considerable hydrogen is produced in recent sediments from the anaerobic decomposition of organic matter.[18] After prolonged incubation under anaerobic conditions, up to 12 percent of the organic content of lacustrine sediments was found[20] to be converted into gaseous products consisting of 65 to 85 percent methane, 3 to 30 percent carbon dioxide, 3 to 22 percent nitrogen and 1 to 3 percent hydrogen.

We have noted the presence of appreciable quantities of hydrogen as well as methane in freshly collected sediments from the Gulf of California.

The anaerobic fermentation of organic matter by bacteria usually results in the liberation of a certain amount of hydrogen. Several species of bacteria are endowed with this ability. The hydrogen is probably liberated in the nascent form which is more reactive chemically than molecular hydrogen.

Effect on Chemical Composition of Sediments

The quality and quantity of organic matter are influenced by bacterial activity. In shallow, well-oxygenated, sandy bottoms most of the organic matter may be completely mineralized, whereas in other regions the organic matter may merely be fermented or reduced. A multiplicity of interrelated factors including the rate of sedimentation, oceanic circulation,[8] pH, redox potential, oxygen content, temperature, the kinds of bacteria present, the nature of the organic matter, the occurrence of specific inhibitors,[32] etc., influence the transformation or organic matter.

Exploratory observations indicate that the oxidizability (by bacteria) of organic matter decreases with core depth, or with the geological age of the material.[41] Because the susceptibility of organic matter to bacterial oxidation is believed to be of importance in characterizing source sediments, efforts are being made to perfect methods for ascertaining the bacterial oxidizability of organic matter in sedimentary materials.

The effect of bacteria on the nitrate, sulfate and carbonate content of sediments as well as their effect on compounds of iron and manganese has already been mentioned.

Besides producing hydrogen sulfide from the reduction of sulfates, this substance may also be produced by the bacterial decomposition sulfo - proteins. There is evidence that under certain conditions sulfates may be reduced to elementary sulfur, and sulfur may be formed by the oxidation of hydrogen sulfide. Thiosulfates may react with hydrogen sulfide to give sulfur, and other chemical reactions might oxidize hydrogen sulfide to sulfur.

Although a high concentration of hydrogen sulfide is toxic for most organisms, many bacteria are able to oxidize it to sulfur. However, most of the bacteria which are known to cause this reaction do so only in the presence of free oxygen or radiant energy.[21]

Particle Aggregation

Many marine bacteria[22] directly or indirectly produce calcareous cements which may tend to accelerate the diagenesis, lithification or consolidation of sedimentary materials. They also produce mucilagenous exudates or capsular substances which adhere to particles or which cause the particles to adhere to the bacteria. The aggregation of particles of clay, silt or sand hastens the rate of sedimentation and influences the structure of the resultant bottom deposits.

The bacteria themselves have an affinity for solids, and a tendency to attach themselves to particulate materials.[23] Filamentous cells weave particles together with an intricate meshwork. The extent of the binding action depends primarily upon the types of microorganisms present and the quality of the organic matter.[24, 25] While there may not be enough living cells present to have any appreciable binding effect, there are probably thousands of times as many dead and partly decomposed cells as the number of living cells which can be demonstrated. An acre-foot of soil or marine mud might contain a ton or more of living microorganisms.

Do Bacteria Produce Petroleum?

It is doubtful if organic matter is converted directly into crude oil by bacterial activity although certain petroleum hydrocarbons are produced by bacteria. However, bacteria in recent sediments do tend to transform organic matter into substances which are more petroleum-like. This they do by reducing the oxygen, nitrogen, phosphorous and sulfur content of organic matter as indicated by the proximate analyses of organic matter from different petroliferous environments:

Source of Organic Matter	Carbon Percent	Hydrogen Percent	Oxygen Percent	Nitrogen Percent	Phosphorus Percent
Marine sapropel . .	49	5	40	3.4	0.8
Recent sediments . .	58	7	34	2.2	0.6
Ancient sediments .	73	9	14	0.9	0.3
Crude oil	85	13	0.5	0.4	0.1

The bacterial reduction of the oxygen, nitrogen, phosphorous and sulfur content of organic matter can be demonstrated in the laboratory. The liberation of fatty acids by the bacterial hydrolysis of fats and vegetable oils has already been mentioned. It would be necessary only to remove two oxygen atoms from the fatty acids to give fairly long-chain hydrocarbons. There is evidence that certain anaerobic bacteria convert fatty acids into non-saponifiable materials consisting of hydrocarbons and sterols.

Investigations are now in progress at Pennsylvania State College, as part of API Research Project 43B, to ascertain whether bacteria isolated from sedimentary materials can deoxygenate or decarboxylate fatty acids. The only hydrocarbon found by Thayer[26] to result from the anaerobic decomposition of fatty acids was methane, but Russian workers[27] report the formation of a mixture of liquid hydrocarbons, balkhashite, from the anaerobic fermentation of fats. According to Ginzburg-Karagicheva[28] anaerobic bacteria produce several kinds of both saturated and unsaturated hydrocarbons. These observations await confirmation by further work.

Pigmented Hydrocarbons

Some bacteria synthesize carotene or other hydrocarbon-like pigments.[29] As much as 0.005 to 0.016 percent of the dry weight of pigmented bacteria may consist of carotene. Smaller quantities of leprotene are produced. When it is considered that approximately one third of the organic matter assimilated by bacteria is converted into bacterial cell substance, it becomes evident that significant quantities of carotene or allied pigments may be produced by bacteria.

It is not inferred that crude oil results from the catalytic or thermogenic cracking of carotenoid pigments. The point is that if bacteria can synthesize such complex hydrocarbons as carotene or leprotene, they may synthesize simpler hydrocarbons.

The occurrence of plant pigments in petroleum has often been cited as supporting evidence for the plant origin of oil.[30] This may be true of certain carotenoid pigments, and quite certainly it is for the chlorophylloid pigments, but cognizance must be taken of the fact that bacteria produce carotene, using either plant or animal organic matter as the energy source.

Methane May Be Polymerized

It is estimated that bacteria produce enough methane to account for all known petroleum deposits if it can be shown that methane is polymerized in source sediments. Some combination of pressure, temperature, time and catalysis may tend to polymerize methane.

Possibly radioactivity activates the polymerization of methane.[17a] Part of API Research Project 43C at the Massachusetts Institute of Technology is concerned with the effect of (a) alpha radiations of radon, (b- deuterons and (c) neutrons upon the transformation of "proto-petroleum." Interesting results have already been achieved.

There are bacteria which utilize methane, converting much of it into bacterial protoplasm. Theoretically, it should be possible for bacteria to polymerize methane to form higher hydrocarbons. An investigation of the possibilities is under way at the Scripps Institution of Oceanography as part of API Research Project 43A.

Bacteria Destroy Petroleum Hydrocarbons

Bacteria have been found in marine sediments, brines, crude oil and soil which can oxidize nearly all kinds of mixtures or purified hydrocarbons which have been tested.[29] In general, long-chain hydrocarbons are oxidized more readily than those of smaller molecular weight, and aliphatic compounds are more susceptible to bacterial oxidation than cyclic or aromatic hydrocarbons. Compounds with unsaturated bonds appear to be attacked more readily than saturated homologs, and iso- or branched-chain compounds appear to be oxidized faster than normal hydrocarbons.

These generalizations are based upon observations on only about 50 different compounds, and may be revised when more highly purified hydrocarbons are subjected to critical tests. Readers are invited to direct the attention of the author to sources of highly purified hydrocarbons which are not generally available.

Numerous species representing half a dozen genera of bacteria are endowed with the ability to utilize certain hydrocarbons. All of them produce carbon dioxide and bacterial protoplasm as end-products of the reactions, and some produce methane or its homologs, organic acids, alcohols or ketones.

Most of the hydrocarbon-oxidizing bacteria require the presence of free oxygen, although other substances such as nitrates or sulfates may serve as the source of oxygen. The ability of bacteria to oxidize petroleum hydrocarbons under anaerobic conditions is being carefully investigated. If hydrocarbons are oxidized anaerobically, the reaction is much slower than under aerobic conditions.

Although the reaction will take place in an aqueous system, the presence of sand, silt, diatomaceous earth or other particulate adsorbents accelerates the bacterial oxidation of hydrocarbons. When mixed with 100 grams of marine sediment, as much as 1 gram of crude oil might be oxidized by aerobic bacteria in 40 days at 70° F.

The hydrocarbon-oxidizing bacteria grow best at temperatures ranging from 65° to 85° F., although some are active at temperatures as low as 32° F., or as high as 130° F. No attempts have been made to cultivate hydrocarbon-oxidizing bacteria at higher or lower temperatures.

Crude Oil Is Attacked by Bacteria

Six different samples of Kern County (California) crude oil supplied to us through the courtesy of General Petroleum Corporation have been found to be attacked by bacteria in an aqueous system. The bacteria grow in the water at the expense of the overlying oil. The reaction is accelerated by the addition of sand.

It is claimed by some[35] that crude oil acts as an "embalming" fluid which provides an aseptic environment for the preservation of biological materials. Unquestionably crude oil from most fields inhibits the activities of many kinds of bacteria, and it is often bactericidal, but crude oil is not universally anti-bacterial. Living bacteria have been recovered from crude oil. Sedimentation ponds and oil-soaked soil are the best places to find large numbers of hydrocarbon-oxidizing bacteria.[29]

While extensive bacterial activity within crude oil seems improbable, bacteria could be active in the water bordering the oil.[42] Work is now in progress to ascertain whether such bacteria could obtain their oxygen requirements from sulfates or from other compounds. If so, the depth to which they could be active would be limited primarily by the temperature, although some crude oils may prove to be toxic. It is desirable to examine petroleum samples, oil-bearing sands, oil shale, oil-well brines and sources sediments from various regions to see if any *per se* provide an aseptic environment.

The continuous action of bacteria upon petroleum may tend to break large molecules of hydrocarbons down into smaller molecules with the formation of increasing quantities of methane, carbon monoxide and carbon dioxide. The latter might be converted into carbonate in a slightly alkaline environment. Aliphatic and olefinic compounds might be preferentially attacked, depending upon the kinds of bacteria present.

HC-Oxidizing Bacteria Inhibited by Hydrogen Sulfide

Concentrations of hydrogen sulfide as small as M/100,000 retard the bacterial oxidation of petroleum hydrocarbons, and ten times this concentration is inhibitory. It is not yet known whether metallic sulfides such as pyrite, for example, are inhibitory. The inhibitory action probably depends upon the ionization of the sulfides which is largely a function of the pH.

Carbon monoxide, heavy metals and other substances inhibit the bacterial oxidation of hydrocarbons in concentrations which might occur in certain source sediments. Perhaps the presence of inhibitory substances or conditions is prerequisite to the accumulation of petroleum hydrocarbons in recent sediments. Various source sediments, oil sands and oil shales are being examined for the presence of inhibitors. It must be taken into consideration that such inhibitors as hydrogen sulfide might be destroyed or inactivated upon exposure to the air. Therefore it is necessary to focus attention upon conditions *in situ.*

Petroleum Hydrocarbons in Nature

Large numbers of hydrocarbons including heptane, pentadecane, hexadecane, eicosane, heneicosane, docosane, tricosane, pentacosane, hexacosane, heptacosane, octacosane, nonacosane, triacontane and pentatriacontane, besides the previously mentioned pigments, occur naturally in certain plants and animals which have been examined.[17a, 33, 34] Sanders[35] relates that 4 to 6 percent of the dry weight of the candeilla plant consists of wax, and 40 to 60 percent of the wax is hentriacontane. The guayule plant and the rubber tree also are rich in hydrocarbons, chiefly isoprene.

If preserved and entrapped, the naturally occurring hydrocarbons of plants and animals would be more than enough to account for the known deposits of petroleum. Perhaps the hydrocarbons have been destroyed in all except certain environments where conditions are inimical to the activity of hydrocarbon-oxidizing bacteria. Although the writer does not believe that all crude oil deposits have had such an origin, preserved hydrocarbons of plant or animal origin might account for some of the oil.

Regardless of their role in petroleum genesis, hydrocarbon-oxidizing bacteria must be taken into account in examining recent sediments for hydrocarbons, or in experiments designed to show how hydrocarbons are formed.[32] The presence of such bacteria may explain why Thayer[26] found no hydrocarbon except methane resulting from the fermentation of fatty acids by mixed cultures. While there is no reason to believe that higher hydrocarbons were produced, it is conceivable that some might have been produced and destroyed simultaneously by hydrocarbon-oxidizing bacteria.

Hydrocarbons in Marine Sediments

The activity of hydrocarbon-oxidizing bacteria may explain why Trask[31] and others have failed to find petroleum hydrocarbons in recent sediments. Even if hydrocarbons were in the sediment *in situ,* they might be destroyed rapidly by the increased bacterial activity which results from the collection and storage of sediment samples.[32]

Recently Dr. Herbert F. Haas working in our laboratory has detected 10 to 20 mgm. of liquid hydrocarbons in 100-gram samples of recent marine sediments. During a few days storage at room temperature much of the oil disappeared, ostensibly due to bacterial activity. The observations are being continued. Finding more oil in some sediments than in others might furnish a clue to the origin of oil.

Microbiological Prospecting for Oil

As might be expected, hydrocarbon-oxidizing bacteria are most abundant in oil- or gas-soaked soil. Several million such bacteria per gram have been found by Haas[29] in crude-oil-soaked soil from Kansas, Louisiana and Texas. They have also been found around leaking natural gas mains[36, 37] in sufficient numbers to alter materially the chemical composition of the surrounding soils.

If volatile hydrocarbons are slowly escaping from subterranean deposits of petroleum they may be oxidized by bacteria in the surface soil which is oxygenated. There may be tests for the bacterial oxidation products of hydrocarbons which are much more sensitive than the conventional geochemical tests. Colorimetric tests which can be rapidly and inexpensively made in the field have been proposed by Blau[38] as a supplementary method of prospecting for oil. The specificity of the tests is yet to be established.

API Research Project 43A

Further information on the mechanism of and conditions requisite to petroleum formation and accumulation may provide important clues to the location of producing horizons. To gain such information is the chief objective of Research Project 43 on the "Transformation of organic matter into petroleum" which is sponsored by the American Petroleum Institute. The Project consists of three closely integrated parts. Bacteriologists, chemists, physicists and geologists at the (A) Scripps Institution of Oceanography, (B) Pennsylvania State College and (C) Massachusetts Institute of Technology are working cooperatively on various aspects of the problem.

"The immediate field of attack will include the occurrence, formation and transformation of hydrocarbons under the influence of bacteria and suitable types of radiations. The organic matter will be selected as far as possible because of its presumed relation to petroleum deposits or their source beds." Since it is generally believed that most petroleum originated in marine environments, the emphasis will be upon marine materials, but terrigenous and lacustrine materials will not be overlooked.

Research Program

Work along the lines summarized in the foregoing pages has been in progress at the Scripps Institution for several years.[39] Impetus has been given to these studies by the sponsorship of the API which, since July, 1942, has been contributing approximately one third of the annual costs. Of far greater importance than the financial assistance is the cooperation with the workers at Pennsylvania State College and MIT and the API Advisory Committee consisting of a score of petroleum geologists who represent the participating companies. The active interest and cooperation of petroleum geologists and production men all over the country can do much to expedite the research work.

Our part of the program, Project 43A at the Scripps Institution, calls for examining recent and older sediments for the presence of bacteria and for environmental conditions which might influence their activities. Laboratory experiments are in progress on the action of microorganisms upon organic matter under conditions designed to simulate those in nature. The effect of various kinds of bacteria upon petroleum hydrocarbons is receiving special attention. We are also responsible for obtaining the necessary samples of sediments and other marine materials for the other two parts of the project.

Need of Special Samples

At the present time it is not feasible to collect cores longer than 10 to 20 feet from the bottom of the sea. Therefore in order to obtain older sediments, attention is being given to oil-well borings in order to define the lower limits of the biosphere. Reports are current of recovering living bacteria from five- to six-thousand foot oil wells but there is some question whether they are adventitious or autochthonous species.

To be useful for bacteriological or biochemical analyses, samples of brines, oil sands, crude oil or cores must be collected *aseptically* and delivered *promptly* to the laboratory. Directions for the collection and shipment of samples may be obtained from Dr. G. C. Gester, Standard Oil Company of California, San Francisco, or from the geological co-director of the project, Dr. W. P. Rand, Gulf Oil Corporation, Pittsburgh.

Further ideas for the *aseptic* collection of samples from oil wells are solicited. If the engineers can collect suitable samples, we can analyze them. It is important to know whether bacteria are merely instrumental in transforming organic matter and affecting environment conditions in recent sediments or if they continue to function at greater depths in older sediments.

Summary and Conclusions

Due to their small size the biochemical activities of enrichment cultures of bacteria are more useful than their morphological appearance for characterizing bacteria. In spite of their small size, bacteria multiply so rapidly and they are so active physiologically that under favorable conditions they may be responsible for chemical reactions of considerable magnitude.

Some of the difficulties of enumerating and identifying the bacterial population of sediments are discussed.

Significant numbers of living bacteria have been found in recent marine sediments. They occur in ocean depths exceeding 3 miles and in core depths as great as 17 feet.

Stratigraphic changes in the organic and sulfate content of cores indicate that the bacteria are active *in situ.* There is evidence that they could be active within wide ranges of temperature, hydrostatic pressure or salinity.

Numerous bacterial species are found in marine sediments which are physiologically versatile, varieties occurring which can attack virtually any kind of organic matter as well as some bacteria which transform certain mineral components of sediments. Most of the bacteria are facultative aerobes which are able to live in either the presence or the absence of free oxygen.

By producing either acids or bases, bacteria influence the pH of sediments which in turn affects chemical reactions, the precipitation of calcium carbonate, the porosity of limestone or the preservation of calcareous fossils.

Bacteria tend to create reducing conditions in marine sediments. Reducing conditions favor the formation of petroleum hydrocarbons and may influence the state of iron or manganese.

Oxygen is depleted from bottom deposits very early in their history by bacterial activity. Most bacteria produce carbon dioxide and some form carbon monoxide. Methane-producing bacteria are widely distributed in the sea.

Significant quantities of hydrogen are produced from the anaerobic decomposition of organic matter.

There are several kinds of bacteria which transform sulfur and its compounds. Sulfates are reduced, sulfur is liberated and hydrogen sulfide is produced, depending upon the bacterial flora and the environmental conditions.

Bacteria may influence the aggregation of particles and the lithification or consolidation of sediments in a variety of ways.

Although bacteria may not produce petroleum directly, they convert organic matter into substances which are more petroleum-like by reducing the oxygen, nitrogen, sulfur and phosphorus content. It is well known that bacteria produce methane and some produce complex pigmented hydrocarbons such as carotene. Bacteria can convert methane into bacterial protoplasm and they may be able to polymerize it to form higher hydrocarbons.

Many bacteria liberate fatty acids from fats and oils. The removal of two atoms of oxygen from the fatty acids would result in the formation of fairly long-chain hydrocarbons.

Some bacteria destroy petroleum hydrocarbons, and these may be responsible for the failure of workers to find petroleum hydrocarbons in recent sediments or in experimental material with mixed cultures. This reaction might occur during the transportation and storage of samples preparatory to their analyses, or it might occur *in situ* in all except certain environments which are unfavorable to the activity of the hydrocarbon-oxidizing bacteria.

Hydrogen sulfide, certain heavy metals and other substances inhibit the activity of hydrocarbon-oxidizing bacteria.

Changes produced by hydrocarbon-oxidizing bacteria in surface soil over subterranean petroleum deposits from which volatile hydrocarbons are escaping may serve as useful indicators in prospecting for oil.

Although unquestionably geophysical and geochemical processes play an important role in petroleum formation, the influence of bacterial activity upon the formation of proto-pertoleum and especially upon environmental conditions must not be disregarded. The extent and depth to which bacteria continue to be active is a problem for further investigation. Complete information may provide further clues to the discovery of oil deposits.

REFERENCES

[1] S. A. Wakeman, "Principles of Soil Microbiology," Williams & Wilkins Co., Baltimore, 894 pp. (1932).

[2] S. C. Prescott and C. G. Dunn, "Industrial Microbiology," McGraw-Hill Book Co., New York, 541 pp. (1940).

[3] C. E. ZoBell, "Studies on Marine Bacteria. I. The Cultural Requirements of Heterotrophic Aerobes," Jour. Mar. Res., **4**, 42-76 (1941).

[4] S. C. Rittenberg, "Bacteriological Analysis of Some Long Cores of Marine Sediments," Jour. Mar. Res., **3**, 191-201 (1940).

[5] C. E. ZoBell, and D. Q. Anderson, "Vertical Distribution of Bacteria in Marine Sediments," Bull. Amer. Assoc. Petrol. Geol., **20**, 258-269 (1936).

[6] C. E. ZoBell, "Microbiological Activities at Low Temperatures With Particular Reference to Marine Bacteria," Quart. Rev. Biol., **9**, 460-466 (1934).

[7] W. P. Larsen, T. B. Hartzell, and H. S. Diehl, "The Effect of High Pressures on Bacteria," Jour. Infect. Dis., **22**, 271-279 (1918).

[8] H. U. Sverdrup, M. W. Johnson, and R. H. Fleming, "The Oceans: Their Physics, Chemistry, and General Biology," Prentice-Hall, Inc., New York, 1087 pp. (1942).

[9] T. Hof, "Investigations Concerning Bacterial Life in Strong Brines," Recueil des Travaux botaniques neerlandais, **32**, 92-173 (1935).

[10] E. Elazari-Volcani, "Studies on the Microflora of the Dead Sea," Thesis published by Daniel Sieff Res. Inst., Rehovoth, Palestine, 120 pp. (1940).

[11] W. W. Smith, and C. E. ZoBell, "Direct Microscopic Evidence of an Autochthonous Bacterial Flora in Great Salt Lake," Ecology, **18**, 453-458 (1938).

[12] M. V. Howard, and M. W. David, "Development of Porosity in Limestones," Bull. Amer. Assoc. Petrol. Geol., **20**, 1390-1412 (1936).

[13] L. F. Hewitt, "Oxidation-Reduction Potentials in Bacteriology and Biochemistry," London County Council, 81 pp. (1933).

[14] L. H. N. Cooper, "Oxidation-Reduction Potential in Sea Water," Jour. Mar. Biol. Assoc., **22**, 167-176 (1937).

[15] C. E. ZoBell, "The Occurrence and Activity of Bacteria in Marine Sediments," Recent Marine Sediments, Amer. Assoc. Petrol. Geol., Tulsa, Okla., 416-427 (1939).

[16] W. H. Pearsall, and C. H. Mortimor, "Oxidation - Reduction Potentials in Water-Logged Soil, Natural Waters and Muds," Jour. Ecol., **27**, 483-501 (1940).

[17] H. A. Barker, "Studies Upon the Methane-roducing Bacteria," Arch. Mikrobiol., **7**, 0-438 (1936).

[17a] B. T. Brooks, "Chemical Considerations Regarding Origin of Petroleum," Bull. Amer. Assoc. Petrol. Geol., **15**, 611-627 (1931).

[18] A. M. Buswell, "Anaerobic Fermentations," Illinois State Water Survey, Bull., **32**, 1-193 (1936).

[19] J. H. Maxson, "Gas Pits in Non-Marine Sediments," Jour. Sed. Petrology, **10**, 142-145 (1940).

[20] R. J. Allgeier, W. H. Peterson, C. Juday, and E. A. Birge, "The Anaerobic Fermentation of Lake Deposits," Int. Rev. d. ges. Hydrobiol. u. Hydrog., **26**, 444-461 (1932).

[21] C. B. Van Niel, "On the Morphology and Physiology of the Purple and Green Sulphur Bacteria," Arch. f. Mikrobiol., **3**, 1-112 (1931).

[22] W. Bavendamm, "Die Mikrobiologische Kalkfallung in der Tropischen See," Arch. f. Mikrobiol., **3**, 206-276 (1932).

[23] S. A. Waksman, and U. Vartiovaara, "The Absorption of Bacteria by Marine Bottom," Biol. Bull., **74**, 56-63 (1938).

[24] J. P. Martin, and S. A. Waksman, "Influence of Microorganisms on Soil Aggregations and Erosion," Soil Sci., **50**, 29-47 (1940).

[25] T. C. Peele, "Adsorption of Bacteria by Soils," Cornell Univ., Agric. Exper. Sta., Memoir 197, 1-18 (1936).

[26] L. A. Thayer, "Bacterial Genesis of Hydrocarbons From Fatty Acids," Bull. Amer. Assoc. Petrol. Geol., **15**, 441-453 (1931).

[27] L. D. Sturm, and S. I. Orlova, "On the Transformation of Fat, Paraffin, and Palmitinic Acid Under the Influence of Microorganisms From the Ala-Kule-Lake," Microbiol. (Russian) **6**, 754-772 (1937).

[28] T. L. Ginzburg-Karagitscheva, "Microflora of Oil Waters and Oil-Bearing Formations and Biochemical Processes Caused by It," Bull. Amer. Assoc. Petrol. Geol., **17**, 52-65 (1933).

[29] H. F. Haas, "The Bacterial Utilization of Petroleum Products and the Production of Carotenoid Pigments," Thesis, Kansas State College, Manhattan, 94 pp. (1942).

[30] B. T. Brooks, "Origins of Petroleum; Chemical and Geochemical Aspects," Bull. Amer. Assoc. Petrol. Geol., **20**, 280-300 (1936).

[31] Parker D. Trask, "Organic Content of Recent Marine Sediments," Recent Marine Sediments, Amer. Assoc. Petrol. Geol., Tulsa, Okla., 429-453 (1939).

[32] C. E. ZoBell, C. W. Grant, and H. F. Haas, "Marine Microorganisms Which Oxidize Petroleum Hydrocarbons," Bull. Amer. Assoc. Petrol. Geol.

[33] A. C. Chibnall, et al., "The Constitution of the Primary Alcohols, Fatty Acids and Paraffins Present in Plant and Insect Waxes," Biochem. Jour., **28**, 2189-2208 (1934).

[34] W. F. Seyer, "Conversion of Fatty and Waxy Substances Into Petroleum Hydrocarbons," Bull. Amer. Assoc. Petrol. Geol., **17**, 1251-1267 (1933).

[35] J. M. Sanders, "The Microscopical Examination of Crude Petroleum," Jour. Inst. Petrol. Tech., **23**, 525-573 (1937).

[36] C. J. Schollenberg, "Effect of Leaking Natural Gas Upon the Soil," Soil Sci., **29**, 261-266 (1930).

[37] H. I. Harper, "The Effect of Natural Gas on the Growth of Microorganisms and the Accumulation of Nitrogen and Organic Matter in the Soil," Soil Sci., **48**, 461-466 (1939).

[38] L. W. Blau, "Process for Locating Valuable Subterranean Deposits," U. S. Patent Office No. 2,269,889, 4 pp. (1942). Chem. Abst., **36**, 3350.

[39] C. E. ZoBell, "Changes Produced by Microorganisms in Sediments After Deposition," Jour. Sed. Petrol.

[40] C. E. ZoBell, "Studies on the Bacterial Flora of Marine Bottom Sediments," Jour. Sed. Petrol., **8**, 10-18 (1938).

[41] D. Q. Anderson, "Distribution of Organic Matter in Marine Sediments and its Availability to Further Decomposition," Jour. Mar. Res., **2**, 225-235 (1940).

[42] C. R. Baier, "Bakteriologische Erdolstudien," Kiel Meeresforschungen, **2**, 149-156 (1937).

Contribution No. 193

Reprinted from Biological Bulletin, Vol. 84, No. 2, 178–186, April, 1943
Printed in U. S. A.

DEVELOPMENT OF THE PRIMARY GONADS AND DIFFERENTIATION OF SEXUALITY IN TEREDO NAVALIS AND OTHER PELECYPOD MOLLUSKS

WESLEY R. COE

(*Osborn Zoological Laboratory, Yale University, and Scripps Institution of Oceanography, University of California, La Jolla* [1])

In connection with studies on the sexual differentiation and changes of sex in several genera of bivalve mollusks the writer has observed certain morphological characteristics of the gonads which seem not to have been reported previously. These concern particularly the types of cells which compose the gonads in the early stages of development and the provisions for the nourishment of the forming gametes. They pertain also to the earliest sexual differentiation of the primary gonia and their transformation into the functional gametes of the primary sexual phase. Some confusion exists in the literature because of failure to interpret correctly the significance of the two types of sexual cells which in young individuals of *Teredo navalis* and other ambisexual mollusks characterize this initial phase of functional sexuality.

Origin and Early Development of Gonad

In all the pelecypods examined the gonads originate from a group of cells situated in the posterior portion of the body, near the visceral ganglion and on the ventral side of the pericardium. As these primordial germ cells multiply they become more or less completely separated into two groups, situated symmetrically on the two sides of the body. By the continued multiplication of their constituent cells each group grows anteriorly in the surrounding mesenchyme or vesicular connective tissue to form the branching system of tubular follicles which characterizes the gonads of the pelecypods (Figure 1).

Two rather distinct types of gonads are found in these mollusks, each type being characteristic of certain genera. In Teredo, Bankia, Mya, Petricola, Barnea and some other members of the order Teleodesmacea the cells of the gonad soon become differentiated into large, vacuolated follicle cells and primary gonia. The follicle cells function as accessory nutritive cells. They occupy most of the space within the tubular follicle, the primary gonia being scattered along the periphery or near the central axis (Figures 1 to 4). In Ostrea, Pecten, Mytilus, Volsella and some other Prionodesmacea, on the contrary, the gonad is composed almost entirely of gonia, with only minute follicle cells (Figures 5, 6). In these forms the gametogenic cells obtain their nourishment directly from the surrounding vesicular connective tissue. An intermediate condition is found in Anomia and some other genera in which the gonadal follicles are associated with vesicular nutritive cells, often arranged in follicles (Figure 7), or they may be composed of follicles with relatively few accessory nutritive cells.

---

[1] Contribution from Scripps Institution of Oceanography, New Series No. 193.

The genital ducts of the adult open into the mantle cavity near the site of the original gonadal primordium or, in some species, into the kidneys and thence to the mantle cavity. When the individual has become sexually mature the branching gonadal follicles may extend throughout the mesosoma and, in the mussels and a few other pelecypods, even into the mantle beneath one or both valves of the shell.

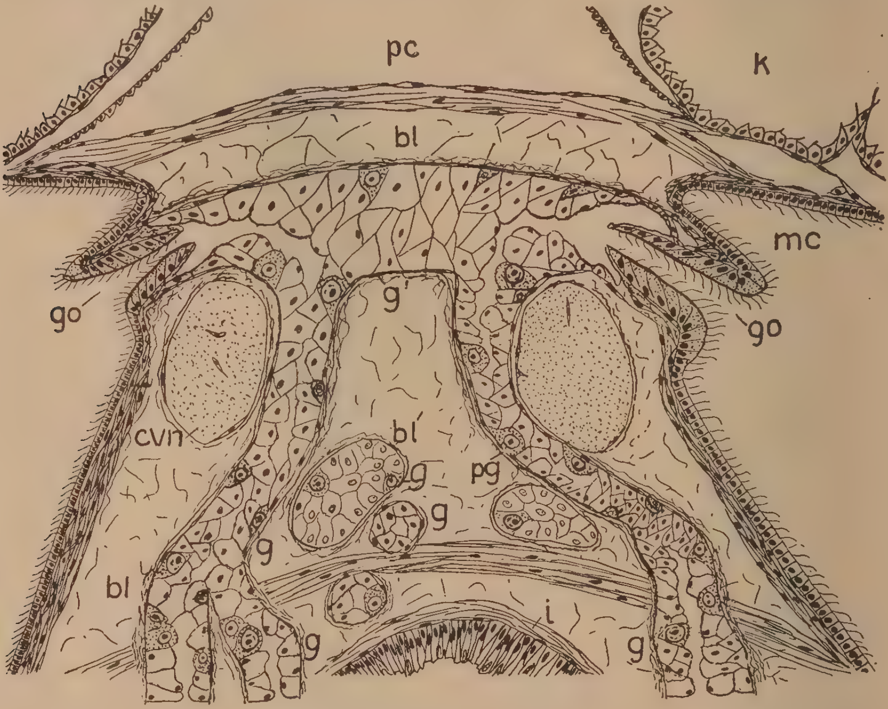


Figure 1. Portion of section through posterior region of body of young *Mya arenaria*, showing early stage in development of the tubular, branching gonads (*g*) composed of large, vacuolated follicle cells with primary gonia (*pg*) scattered along the periphery; *bl*, vesicular connective tissue; *cvn*, cerebro-visceral nerve cords; *go*, genital openings; *k*, kidney; *i*, intestine; *mc*, epibranchial chamber; *pc*, pericardial cavity. (Modified from Coe and Turner, 1938.)

Sexual Differentiation

In *Teredo navalis*, which normally experiences alternating male and female phases of functional sexuality, the gonadal primordia consist of groups of cells, all of which are identical in appearance. But as soon as the primordia begin to branch out to form the tubular follicles, as described in a preceding paragraph, two distinct types of cells become distinguishable. At the end of each growing follicle there is a cap of undifferentiated nuclei in a continuous mass of cytoplasm (Figures 2, 4). As growth proceeds some of these nuclei become ecnlosed in large, vacuolated cells which function thereafter as accessory nutritive cells. A smaller number retain a larger portion of the terminal cytoplasm and become the primary gonia. As the follicle increases in length these primary gonia are left behind on the periphery of the follicle cells which otherwise fill the entire follicle (Figures 2, *A*, 4). The primary gonia multiply slowly as the follicles increase in length.

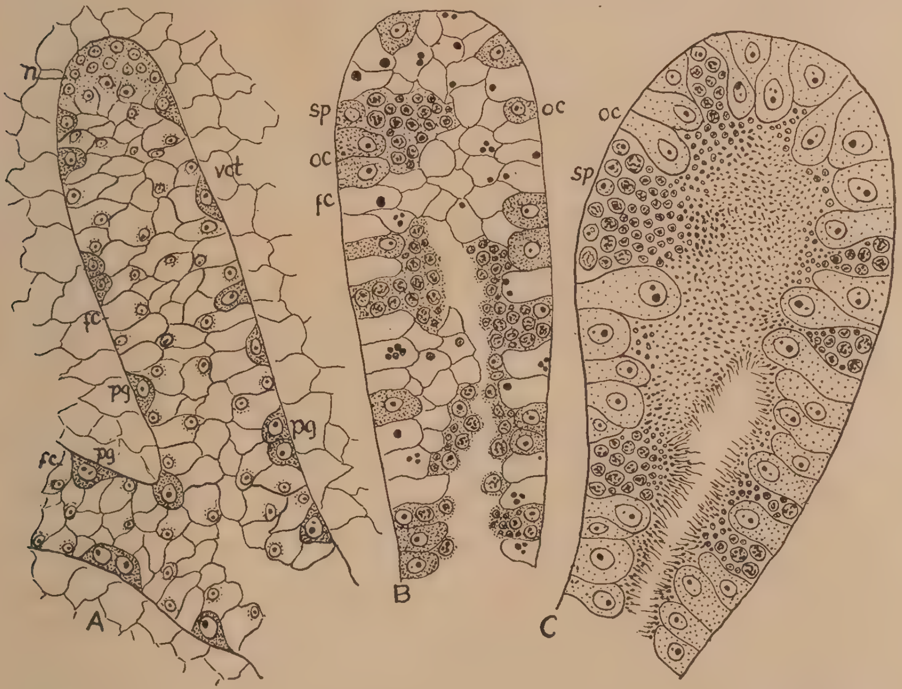


FIGURE 2. Development of primary gonad in *Teredo navalis*. *A*, sexually undifferentiated branching follicle, composed of large, vacuolated follicle cells (*fc*) with primary gonia (*pg*) scattered on the periphery; *n*, terminal cap of undifferentiated proliferating nuclei; *vct*, vesicular connective tissue. *B*, later stage with differentiated ovocytes (*oc*) and spermatogenic cells (*sp*) derived from the primary gonia; *fc*, remaining follicle cells with disintegrating nuclei and products of abnormal spermatogenic cells. *C*, follicle of primary male phase, with spermatogenic cells (*sp*) and spermatozoa filling the lumen and large ovocytes (*oc*) on the periphery.

No sexual differentiation can usually be detected in the primary gonia until the young teredo has attained a length of about 10 mm. In the warm season of the year, when the water is above 18 degrees C., this length may be reached within three to four weeks after entering the wood. Dwarfed individuals become sexually differentiated at a smaller size but at approximately the same age.

The spermatogonia are recognized as soon as the primary gonia begin proliferation into groups of nuclei within a cytoplasmic syncytium. These soon form primary spermatocytes with characteristic phases of synapsis (Figure 2, *B*).

Approximately half the total number of primary gonia become activated in this manner. An equal number differentiate into ovogonia and their resulting ovocytes.

Each of the rapidly growing ovocytes remains attached to the wall of the follicle until fully mature. The spermatogenic cells, on the contrary, multiply rapidly and soon fill the axis of the follicle (Figure 2, *B*). The follicle cells have meanwhile begun to undergo cytolysis and disintegration. Their nuclei first become pycnotic and are later cytolyzed. The collapse of the follicle cells forms a central lumen in the follicle and in this cavity spermatogenesis is completed (Figure 2, *C*).

With the continued growth of the ovocytes and a vast increase in the number of spermatogenic cells the follicles become greatly distended and the former lumen packed with spermatozoa (Figure 2, *C*). This is a typical hermaphroditic gonad but since the spermatozoa will usually become ripe and will be discharged before the ova are fully mature the individual is properly recognized as being in the primary, or protandric, or ambisexual male phase.

If all individuals experienced the same aspects of sexual differentiation there could be no difference of opinion as to their status, but an examination of the gonads of more than 3000 individuals shows wide variations in the proportion of spermatogenic and ovogenic cells present. This is true not only for different individuals but for different follicles of the same individual (Coe, 1933, 1934a). Photographs of some of these variations are shown by Coe (1933).

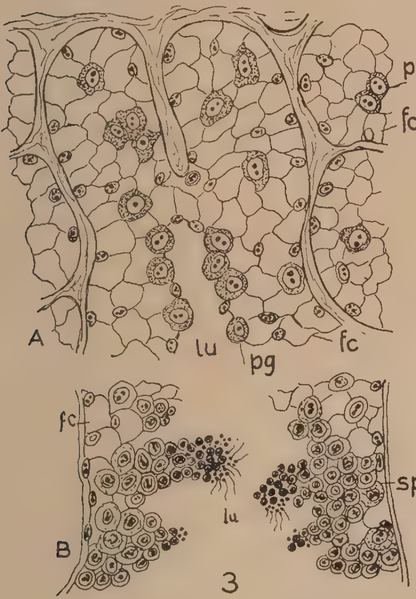
At one extreme are the so-called true males, in which the ovogonia, if present, fail to produce ovocytes. At the other extreme there are proterogynic individuals which form ovocytes exclusively in their first functional sexual phase, the usual primary male phase being aborted or inhibited (Coe, 1935). In exceptional individuals both spermatogonia and ovogonia are activated simultaneously, producing functional hermaphrodites. Some of these are capable of self-fertilization (Coe, 1941).

Both the true males and the proterogynic individuals must be considered as exceptions to the great majority of the population in which each individual normally functions first as male and later as female. The female phase will be followed by a second series of male and female phases if the life of the individual be sufficiently prolonged. This alternation or rhythm of sexual phases may be considered as characteristic of the species, although the mortality is usually very high before even two of the phases have been completed (Coe, 1934a).

Furthermore some of the typically protandric individuals approach more or less closely the true males in having relatively few ovocytes in the gonads. They retain the male phase longer than others which bear a closer resemblance to the proterogynic individuals in the appearance of the primary gonads (Coe, 1934a, 1936).

Because of this variability in the expression of ambisexuality, as well as associated differences in the time necessary to complete any one of the sexual phases, the proportion of individuals in each phase found in any sample of the population would seem to have less significance than has sometimes been assumed (Grave and Smith, 1936; Coe, 1936). It is obvious that if in such a sample twice as many individuals are found in one sexual phase as in another it may be merely indicative that the one phase lasts twice as long as the other. It might also indicate that some individuals are older than others, since the sexual phases appear in sequence during the life of the individual, with the exceptions already mentioned.

During the summer months there will ordinarily be fully three to five times as many individuals in the primary male phase as in the female phase at the first spawning period. This may occur as early as six weeks after metamorphosis. Three or four weeks later, after the change of sexual phase, the same individuals may be expected to show an approximation to a reversed proportion of male and female phases. There will be many deviations, however, since, as already mentioned, the primary sexual phase will be more prolonged in some individuals than in others and some will change quickly to the third (ordinarily male) phase. In the meantime younger members of the population will be functioning in the primary male phase.



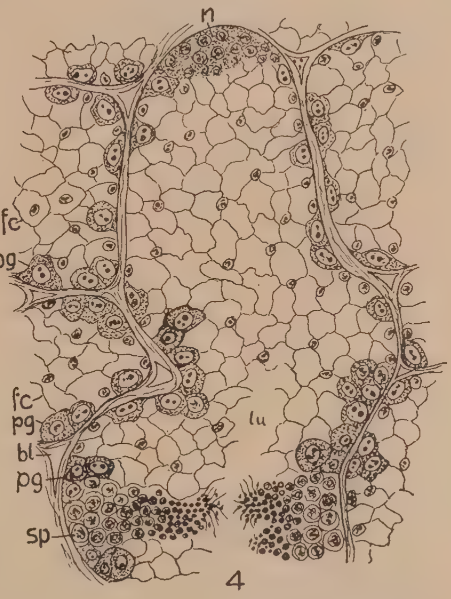
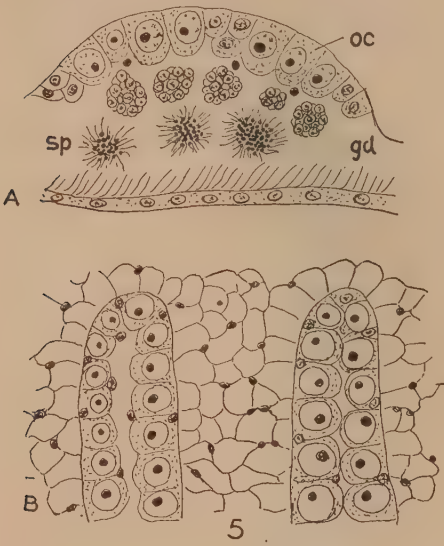


FIGURE 3. Portions of primary gonads of *Petricola pholadiformis*. *A*, sexually undifferentiated stage; follicle composed mainly of vacuolated, nutritive follicle cells (*fc*) with primary gonia (*pg*) along the central axis or lumen (*lu*). *B*, later stage in young male; most of the follicle cells have been cytolyzed to supply nourishment for the proliferating spermatogenic cells (*sp*).

FIGURE 4. Development of primary gonad in *Mya arenaria;* central follicle with terminal cap of undifferentiated nuclei (*n*), from which both follicle cells (*fc*) and primary gonia (*pg*) originate; lower portion of follicle shows later stage in which the follicle cells are being replaced by spermatogenic cells (*sp*), with spermatozoa adjacent to the lumen (*lu*). Note blood vessels (*bl*) and small amount of connective tissue between follicles.

The proportion of individuals in which the first sexual phase is female is thought by Grave (1942) to be larger than formerly reported (Coe, 1936) but the data presented do not seem to justify such a conclusion.

The following scheme, modified from Coe (1935), indicates such variations in the sequence of sexual phases as are inferred from a study of more than 3000 individuals at various ages. It is impossible to follow these phases in any single individual because the teredo dies soon after it is removed from the wood for examination. The presence of larvae in the gill chambers, however, is sufficient proof that the preceding sexual phase of the gonad was female.



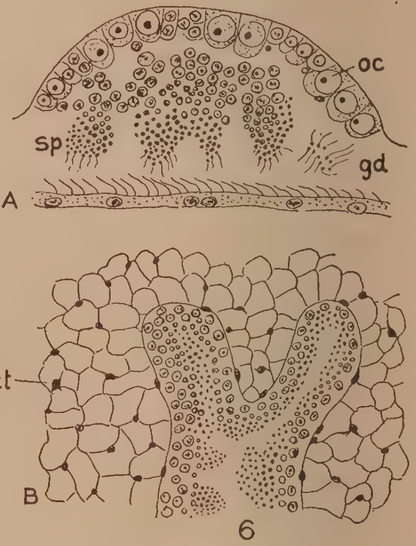


FIGURE 5. *Ostrea lurida.* *A*, portion of primary gonad showing typical ambisexual male-phase condition, with peripheral layer of ovocytes (*oc*) and developing sperm balls (*sp*) in the lumen; *gd*, ciliated genital duct. *B*, terminal portions of two follicles of secondary gonad in early female phase surrounded by much vesicular connective tissue (*vct*).

FIGURE 6. *Ostrea virginica.* *A*, portion of primary gonad in ambisexual condition. *B*, terminal portion of follicle surrounded by much vesicular connective tissue in early male phase of adult; letters as in Figure 5.

1. True male phase (exceptional) . . . Second male phase . . .
2. Ambisexual male phase . . . First female phase . . . Second male phase . . . Second female phase (if life be sufficiently prolonged).
3. Functional hermaphroditic phase (exceptional) . . . Male or female phase . . .
4. Female phase (exceptional) . . . First male phase . . . Second female phase . . .
5. Female phase (exceptional) . . . Second female phase (?) . . .

Many other pelecypods resemble the teredos in having the primary gonads composed largely of nutritive follicle cells. In some of these, of which *Petricola pholadiformis* may be taken as an example, the primary gonia are scattered along the central axis of the follicle (Figure 3). In others, as *Mya arenaria* (Coe and Turner, 1938), they are distributed along the periphery (Figure 4). In both cases the follicle cells are cytolyzed during gametogenesis. With the exception of an occasional hermaphrodite, all individuals of both species are strictly unisexual and the two sexes are approximately equal in number.

A different type of gonad is found in both the larviparous and oviparous oysters (Ostrea), in scallops (Pecten), mussels (Mytilus, Volsella), and numerous other bivalves. In these the gonads are composed almost entirely of gametogenic cells which receive their nourishment directly from the sourrounding vesicular connective tissue. The follicle cells are few and small (Figures 5, 6). As the gonadal follicles increase in size the surrounding nutritive connective tissue is utilized.

The larviparous oysters, of which *Ostrea lurida* may serve as an example, exhibit changes in sexuality during life, the sequence of sexual phases being similar to those mentioned for *Teredo navalis* (Coe, 1934). In the oviparous oysters, as *O. virginica*, there is likewise a strong tendency toward protandry, since about 70 per cent of the young individuals first function as males (Coe, 1938). The primary gonad usually contains antecedent cells of both sexual types (Figure 6, *A*). Functional hermaphroditism occurs occasionally and a few individuals change from the male to the female phase during their first spawning season. Thereafter the individual functions in one sexual phase or the other during all of each spawning season, but not infrequently the sexual phase changes between two spawning seasons (Burkenroad, 1937; Galtsoff, 1938; Coe, 1938; Loosanoff, 1942). In

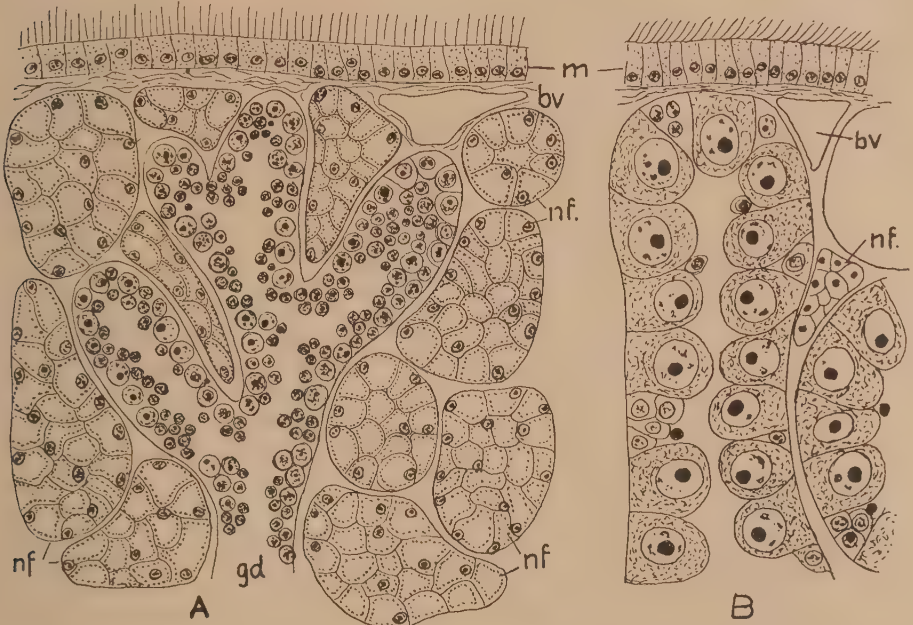


FIGURE 7. *Anomia simplex*. *A*, portion of primary gonad surrounded by nutritive tissues (*nf*). *B*, follicles of mature ovary in which the nutritive tissues (*nf*) have been almost assimilated; *bv*, blood vessels; *m*, mantle; *gd*, genital duct.

Venus the sexes are separate, following a juvenile, usually protandric, sexual phase (Loosanoff, 1937).

Gonads in some respects intermediate between the other two are found in Anomia and in some other genera. Here the gonadal follicles, which are composed almost entirely of gametogenic cells, are surrounded by nutritive tissues of similar configuration (Figure 7). As in the other types of gonads the nutritive tissues are utilized during the course of gametogenesis (Figure 7, *B*).

SUMMARY

Two more or less distinct types of primary gonads are found in bivalve mollusks. The simplest type occurs in several families of the order Prionodesmacea, where the profusely branching follicles on each side of the body are composed mainly of gametogenic cells and each follicle is nourished by the surrounding mesenchyme or vesicular connective tissue.

In the second type, found mainly in the order Teleodesmacea, the branching follicles of the primary gonads are composed principally of large, vacuolated follicle cells of a nutritive nature, the primary gonia being scattered along the central axis or on the periphery. These nutritive cells are cytolyzed during gametogenesis.

In some bivalves intermediate conditions are found, with associated gametogenetic follicles and nutritive tissues.

In the ambisexual or hermaphroditic species, as *Teredo navalis*, the primary gonia are early differentiated into the two sexual types. Of these the spermatogonia usually, but not invariably, proliferate and complete gametogenesis in advance of the ovogonia, giving each follicle the appearance of a spermary with a layer of ovocytes on the periphery. As a general rule the ovocytes do not become fully mature until after the discharge of the spermatozoa. There are many variations in the relative proportion and time of spawning of the two types of gametes, however, and in exceptional cases the gonad is almost exclusively of the male or of the female type. Most individuals function first in the male phase, then change to the female phase, while some individuals experience in addition a second sequence of male and female phases. Less frequently a primary female phase precedes the first male phase. Individual differences in the combinations of the modifying hereditary sex factors are presumably responsible for most of these variations.

Each species studied, except those that are strictly unisexual, shows some variations in the sequence of male and female or functionally hermaphroditic phases.

LITERATURE CITED

Burkenroad, M. D., 1937. The sex-ratio in alternational hermaphrodites, with especial reference to the determination of sexual phase in oviparous oysters. *Jour. Marine Research*, **1**: 75–84.

Coe, W. R., 1933. Sexual phases in Teredo. *Biol. Bull.*, **65**: 283–303.

Coe, W. R., 1934. Alternation of sexuality in oysters. *Am. Nat.*, **68**: 236–252.

Coe, W. R., 1934a. Sexual phases in the pelecypod mollusk Teredo. *Science*, **80**: 192–193.

Coe, W. R., 1935. Sequence of sexual phases in Teredo, Ostrea and Crepidula. *Anat. Rec.*, Suppl., **64**: 81.

Coe, W. R., 1936. Sequence of functional sexual phases in Teredo. *Biol. Bull.*, **71**: 122–132.

Coe, W. R., 1938. Primary sexual phases in the oviparous oyster (Ostrea virginica). *Biol. Bull.*, **74**: 64–75.

Coe, W. R., 1941. Sexual phases in wood-boring mollusks. *Biol. Bull.*, **81**: 168–176.

Coe, W. R., and Harry J. Turner, Jr., 1938. Development of the gonads and gametes in the soft-shell clam (Mya arenaria). *Jour. Morph.*, **62**: 91–111.

Galtsoff, Paul S., 1938. Sex change and physiological intersexuality in Ostrea virginica. (Abstr.) *Anat. Rec.*, Suppl., **72**: 42.

Grave, Benjamin H., 1942. The sexual cycle of the shipworm, Teredo navalis. *Biol. Bull.*, **82**: 438–445.

Grave, Benjamin H., and Jay Smith, 1936. Sex inversion in Teredo navalis and its relation to sex ratios. *Biol. Bull.*, **70**: 332–343.

Loosanoff, Victor L., 1937. Development of the primary gonad and sexual phases in Venus mercenaria Linnaeus. *Biol. Bull.*, **72**: 389–405.

Loosanoff, Victor L., 1942. Seasonal gonadal changes in the adult oysters, Ostrea virginica, of Long Island Sound. *Biol. Bull.*, **82**: 195–206.

Contribution No. 194

Reprinted from Biological Bulletin, Vol. 84, No. 2, 187–194, April, 1943
Printed in U. S. A.

AN IMPROVED METHOD OF ASSAYING MELANIN IN FISHES[1]

F. B. SUMNER AND PETER DOUDOROFF

(*Scripps Institution of Oceanography of the University of California, La Jolla*)

Some five years ago, the authors described a method of evaluating the melanin content of fishes (Gillichthys) kept under various experimental conditions, along with some preliminary results obtained by the use of this method (Sumner and Doudoroff, 1937). In the following year we published a brief account of similar experiments, conducted upon another teleost, *Gambusia affinis* (Sumner and Doudoroff, 1938). In these last it was found advisable to revise our earlier procedure in important ways.

Despite certain obvious shortcomings in the technique employed in those earlier studies, interesting quantitative relations were shown to obtain between the melanin extracted from our fishes and the optic stimuli to which they had been subjected. Whatever the precise nature of these relations, there could be no reasonable doubt that fishes (of at least two species) which had lived for some weeks upon black or dark gray backgrounds had a much higher melanin content than fishes kept upon various paler backgrounds. This was in full agreement with experiments involving the counting of melanophores, through which it had been shown that both the number of these cells per unit area of skin and the amount of melanin per cell may be greatly influenced by the background.[2] Much remained to be learned, of course, regarding the details of these relations.

In quantitative studies of melanin, gravimetric determinations seem to be ruled out by the impossibility of isolating this substance without sacrificing a large proportion of it during the process of isolation. Various methods of obtaining pure melanin have been developed by chemists whose object has been to purify it for chemical analysis, rather than to measure accurately the amount present in a given sample of tissue (e.g., Gortner, 1910, 1911; Salkowski, 1920; Heinlein, 1924).

Colorimetric methods were consequently resorted to early in our endeavors to assay melanin. We know of only two previous workers (Kudo, 1922; Vilter, 1931) who had attempted the measurement of changes of melanin resulting from experimental optical conditions, at least in fishes and amphibia. Recently, however, Dawes (1941) has published the results of some interesting experiments upon *Rana temporaria*.[3] His findings, so far as the effects of black and white backgrounds are concerned, fully confirm our own. Considering the facts just stated, however, it is obvious that Dawes is over-enthusiastic in his claim that his work "affords conclusive evidence for the first time that prolonged exposure of one of the lower vertebrates to . . . black background results in a marked increase in the melanin content of the skin."

---

[1] Contributions from the Scripps Institution of Oceanography, New Series, No. 194.

[2] The work of ourselves and others in this field has been summarized by Sumner (1940b).

[3] Dawes's technique involved peptic digestion of the frogs' skins.

The employment of colorimetric methods in "melaninometry" is rendered possible by the solubility of this substance in dilute alkali.[4] Admittedly these methods involve some rather serious difficulties. Some of the primary requirements in the preparation of such a melanin solution are: (1) to retain without loss and unaltered the melanin contained in each sample of material; (2) to eliminate all cloudiness and obtain a perfectly clear solution; (3) to eliminate any colored materials other than melanin; (4) to avoid the production of pseudo-melanins ("melanoidins") which sometimes result from the action of strong acids upon proteins.

The procedure outlined below is the outcome of experimentation by the authors, which was commenced in 1935. While it is empirical in many details, we believe that the various steps are theoretically justifiable. Many of these steps are based upon the methods of previous workers, but the combination is ours.

Comparison with our earlier report (1937) shows that somewhat extensive changes have been made in this procedure. Since little melanin is contained in the more massive tissues of the body (muscles and viscera), the presence of which increases the difficulty of preparing clear solutions, it was decided to exclude these tissues; hence paragraphs 4, 5, and 6 of our revised procedure (cf. Sumner and Fox, 1935).[5] Again, peptic digestion, followed by centrifuging, was replaced in our revised technique by boiling in 6 per cent HCl, followed by dialyzing (paragraphs 22 to 25).

Our most recent procedure follows:

(1) The fishes (unfed for at least two days) were killed in chloroform vapor; (2) dried on paper towels; (3) each individual measured, and each lot (those combined to form a single sample) weighed; (4) dipped into boiling distilled water $\frac{3}{4}$ minute, then cooled in cold water; (5) the skin was removed from the entire body and head and placed (along with fins) in distilled water; (6) water warmed to 60° C. and beakers placed in oven at that temperature for one hour (to remove gelatin); (7) 95 per cent alcohol substituted for water, changed once, material bottled and kept in the dark, sometimes as long as five or six weeks; (8) alcohol poured off and material subjected to Soxhlet fat-extration (in 150 cc. thimbles) for three hours, using 250 cc. of alcohol-ether mixture (2 : 1); (9) material left in thimbles and dried in oven at 60° for several hours; (10) decalcified in one per cent HCl for one hour at 60° (5 cc. per gm. of original weight of fish, *not of skin*); (11) acid filtered off through no. 2 sintered-glass filter, residue washed with 500 cc. distilled water; (12) residue from filter hydrolyzed by boiling one hour in 0.2 per cent NaOH (5 cc. per gm. of original weight of fish) in three-liter flask, under reflux condenser; (13) resulting mixture filtered through no. 2 glass filter, to remove undissolved impurities; (14) filtrate, when of sufficient volume, divided into two parts ("a" and "b") to be run separately as checks; (15) HCl added, drop by drop, to each lot, until neutral point is just past and a precipitate forms which includes the melanin (important that approximately the same pH

---

[4] Strictly speaking, these are not molecular solutions, but very fine colloidal suspensions. They may be very clear, however, and there is little or no Tyndall-effect unless the suspensions are highly concentrated.

[5] As we were primarily interested in changes in the external pigmentation (that of the skin), we had no object in retaining the considerable amount of melanin contained in the peritoneum or in the eyes.

is reached for all samples); (16) material (liquid and precipitate) transferred to centrifuge tubes (approximately 100 cc. capacity), centrifuged 15 minutes at approximately 1760 r.p.m., liquid[6] poured off (precipitate adheres closely to bottom of tube); if all material from one sample is not, at first, contained in a single tube, it is combined into one tube and re-centrifuged; (17) 95 per cent alcohol carefully poured onto precipitate in tubes (4 cc. per gm. of original weight of fish), left at least ½ hour; (18) this alcohol removed by suction (using a glass tube, finely drawn out), and replaced by same amount of absolute alcohol, left at least one hour; (19) alcohol again removed and ether (the same quantity) substituted, precipitate thoroughly stirred, left at least one hour; (20) materials centrifuged ½ hour, and ether removed by suction; (21) dried in oven at 60°; (22) dissolved (while still in centrifuge tubes) in 0.2 per cent NaOH (1 cc. per gram of original weight of fish); (23) equal volume of 12 per cent HCl added, making a concentration of (nearly) 6 per cent acid; (24) transferred to flasks and boiled one hour under reflux condensers; (25) liquid, with precipitate, transferred to celloidin ("Parloidin") dialyzers, suspended in one-liter beakers of distilled water. Left at least three hours, during which water is changed five times; (26) material poured (and rinsed) from each dialyzer into graduated cylinder, enough 5 per cent NaOH added to make a 0.2 per cent solution, when liquid has been brought to final required volume (3 cc. per gm. of original weight of fish); (27) solutions returned to flasks and boiled for one hour under condensers (if necessary the condensers are left off for a time and liquid evaporated sufficiently to bring the volume down to *somewhat less* than the required final volume, to allow for rinsing); (28) solutions returned to graduates and enough distilled water added to bring them as nearly as possible to required volume (3 cc. per gram of fish); (29) passed through no. 2 sintered-glass filters, previously dried in oven (no material loss of melanin by adsorption is found to take place in a filter of this grade of porosity).

The melanin "solutions" are now satisfactorily clear and ready for direct colorimetric reading by transmitted light. This is obviously more satisfactory than our earlier, more involved procedure (1937).

The colorimeter employed in these studies (the Ives Tint-Photometer) has been employed by the senior author and collaborators for a number of years in several lines of investigation. Since it has already been described rather fully (Sumner and Fox, 1933, and earlier), no detailed account is called for. One beam of light, reflected from a block of white magnesium carbonate, is passed through an absorption-cell containing the solution under examination, another beam through a neighboring cell containing distilled water. An adjustable diaphragm controls the amount of light emerging from the latter cell so that the intensity of the beams emerging from the two cells can be brought to equality. Before reaching the eyes of the observer however, both beams are passed through one of a series of color filters. Using each of these filters in turn, the two halves of the field are balanced and the readings recorded. For the present studies, three filters have been employed: red, green, and blue.[7] It is evident that in the use of this instrument high readings represent low melanin values and vice-versa. The highest (100 per cent) readings are obtained from pure water.

---

[6] This liquid is ordinarily clear and of a pale straw-color, quite different from that of a diluted melanin solution. Solutions from "black" fishes (i.e., ones from black background) were found, however, to be about 80 per cent more deeply tinted than ones from "white" fishes when several pairs of samples were compared. At most, the coloration is feeble.

These successive readings constitute the primary data upon which our assays of melanin are based. Each observer repeats his readings several times. When two observers work in collaboration, their readings are averaged.[8]

For the interpretation of these figures for color-values obtained with the tint-photometer it is necessary to prepare a set of curves based upon melanin solutions of known concentration. Through the kindness of the late Professor R. A. Gortner, of the University of Minnesota, we obtained a sufficient quantity of purified melanin, prepared by him from the skin and other tissues of the "silky" fowl. Curves based upon tint-photometer readings of a series of dilutions of a solution (.0667 gm. per 100 cc. 0.2 per cent NaOH) are shown in Figure 1. These are the curves which have been employed by the senior author in deriving the values reported for Girella and Fundulus in this issue (pp. 195–205). The

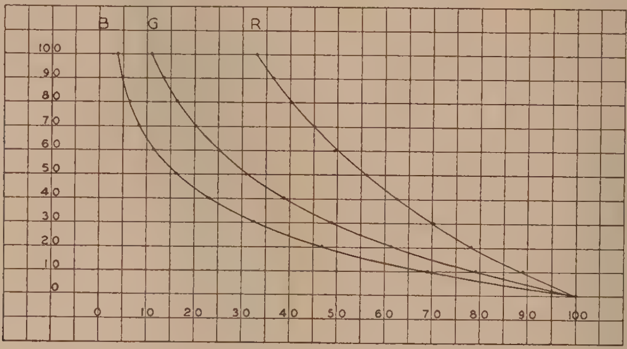


FIGURE 1. Tint-photometer readings of melanin solutions of various concentrations. The highest concentration ("100%") was prepared by boiling dried melanin for three hours in 0.2 per cent NaOH solution in the proportion of 0.667 gms. to 100 cc. This was variously diluted with NaOH solution. Abscissas = tint-photometer readings; ordinates = dilutions (expressed as percentages of "100%" solution).

---

[7] Specifications for these filters, taken by means of the Bausch & Lomb Visual Spectrophotometer are as follows:

		mμ
Red	Maximum transmission (70% ±)	760–650
	Lower limit (1% transmission)	602
Green	Maximum (18% ±)	530–520
	Upper limit (1%)	610
	Lower limit (1%)	475
	Points of half transmission	500, 565
Blue	Maximum (31% ±)	460–450
	Upper limit (1%)	515

(The blue has been referred to as "blue-violet" in our earlier publications.)

[8] There was, not unexpectedly, a personal equation in these readings. In the case of the last ten samples studied by us, melanin values based upon the readings of the senior author were 1.7 per cent higher than those obtained by the junior author. The mean differences, regardless of sign, averaged 2.0 per cent.

melanin values therein presented are expressed in milligrams per gram of the original weight of the fish.

Allowance must be made for the probability that melanins derived from different organisms are not all chemically identical. Hence, these figures for fish melanin, based upon comparisons with the melanin of a bird, may be no more than rough approximations. In our present studies, however, we are chiefly concerned with relative rather than absolute values, our main object being the discovery of relations among our various experimental groups.

We feel justified in basing our melanin titer upon the weight of the fishes, rather than upon the surface area of the skins, for the reason that the skin of an animal has a third dimension, thickness, which probably varies roughly in proportion to its other dimensions. (It is obvious that the wet weight of the scraped-off skins would be of no significance for this purpose.) It is of interest, however, that when the melanin content, *per square of body length*, is computed (in the absence of any actual measurements of surface areas), the essential relations among the various experimental lots remain unchanged.

Previous writers have called attention to the production of dark "melanoid" substances through the action of strong acids upon proteins. These would obviously interfere with colorimetric measurements of melanin. That boiling with 6 per cent HCl can have had no such effect upon our material seems to be proved by a special test which was made for this purpose. Twelve goldfishes, very nearly devoid of melanin in their skins, were subjected to our standard procedure in three lots of four fishes each. These agreed in yielding solutions hardly tinted enough to permit of readings with the tint-photometer. The slight coloration was about 2 per cent of that of the "100 per cent" standard melanin solution (Figure 1). Moreover, in six cases, one to two additional hours of boiling in HCl (step 24, p. 189) resulted in a slight mean *loss*, rather than increase in the melanin titer, though this difference was of doubtful significance.

The same cannot be said, however, of extending the time of boiling in sodium hydroxide. Six samples which were boiled for three hours gave melanin values averaging 7.4 per cent lower than portions of the same solutions which were boiled the usual one hour.

Furthermore, as we have reported earlier (Sumner and Doudoroff, 1938) alkaline solutions of melanin invariably deteriorate (become paler) at ordinary room temperatures, even when kept in the dark. Thus a set of three samples from black-adapted fishes underwent a mean apparent loss in melanin content of 9 per cent in 24 hours, 18.6 per cent in five days, and 24.3 per cent in 12 days. These figures are based upon the means for the three color filters. Actually, there were wide differences between the figures for loss, when based upon readings with the different filters. Thus, at the end of 12 days, the figures seem to show a decrease of 33 per cent, if readings with the red filter alone are considered; only 16 per cent if the blue ones alone are considered. This obviously implies a change in the color as well as in the density of our solutions.

Readings taken of our "standard" melanin solutions at the end of 12 days presented much the same picture. In the "70 per cent" solution[9] (corresponding approximately to that derived from black-adapted Girella) the seeming loss in melanin concentration, when based upon the mean of readings with the three filters, was 20.5, a figure not far from that shown by the solutions of fish melanin. Here, too, the separate figures, based upon the three filters, ranged from 27 per cent (red) to 15.7 per cent (blue).

---

[9] Meaning 70 per cent of the concentration of the strongest solution (taken as 100 per cent) upon which Figure 1 is based.

Some further optical properties of a melanin solution, both when freshly prepared and after standing in the dark for 12 days, are portrayed in Figure 2. This is based upon readings with a Bausch and Lomb Visual Spectrophotometer of a solution having the same concentration as the "100 per cent" solution of Figure 1. The "curve" of transmission, at least for the freshly prepared solution, turns out to be a nearly straight line, with a practically uniform gradient from blue through red.

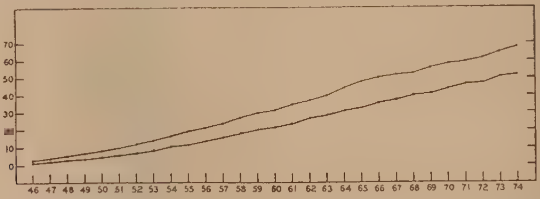


FIGURE 2. Curves of transmission of various wave-lengths by "100%" solution of purified melanin (see legend for Figure 1). Lower curve = freshly prepared solution. Upper curve = same solution after 12 days. Abscissas = wave-lengths at 10-mμ intervals; ordinates = percentages of transmission in comparison with distilled water.

When a fresh "70 per cent" solution is compared with a 12-day-old solution of the same original strength, we find an increase of 22 per cent in the reading with the red filter, an increase of 41 per cent in the reading with the blue. This might be construed as an increase in transmission toward the blue end of the spectrum. It must be remembered, however, that with increasing dilution, the transmission of the various wave-lengths through a colored solution tends to equalize, and that the least transmitted wave-lengths (in this case blue) will increase much more rapidly than the more transmitted ones (in this case red). In passing from a "70 per cent" to a "51 per cent" melanin solution, for example, we find an increase of 22 per cent in the "red" reading, and an increase of 89 per cent in the "blue" reading. Thus the much smaller increase in the transmission of blue rays through the 12-day-old sample, in comparison with the diluted fresh sample, denotes a shift toward the red on the part of the former. The figures upon which Figure 2 is based likewise reveal a greater relative transmission of the red rays than would result from simple dilution.

This lability of melanin solutions makes it necessary to employ boiling periods of uniform length in preparing them, and to make the photometric readings as soon as possible after the solutions are prepared—three or four hours at most. The unavoidable losses are thus kept constant, and the figures for the various lots may be regarded as strictly comparable.

A chief reason for our dissatisfaction with our earlier procedures (1937, 1938) was the frequent appearance of considerable differences in the yield of samples from which identical results had been expected. So far as such unexpected differences related to different lots of fishes having the same experimental history, there was, however, no real ground for concern. Later studies of melanophore density (Sumner, 1940, *a* and *b*) have emphasized the enormous variability in this respect within each of the experimental lots. There was considerable overlapping, for example, between the frequency distributions of melanophore numbers in even the "black" and "white" series of Lebistes.

Of more serious import are differences in results obtained from portions of the same sample of material which, after division, have been subjected to identical treatment. Such discrepancies, which were earlier encountered to a discouraging degree, have been largely, though not wholly, eliminated in our later studies. Comparisons of figures for "a" and "b" portions of the same sample (step 14, above) give us something of an index of the degree of constancy of our results. In 34 cases in a recent study, in which readings from two such portions were obtained, the mean difference between these portions was 3.2 per cent. This figure, it is true, indicates only a moderate degree of precision, but it is small compared with most of the experimentally produced differences upon which our conclusions are based. In 28 of these 34 cases, the differences between our "a" and "b" sub-samples were under 5 per cent. In the remaining six, they were 5 per cent or more, the largest figure being 12.3 per cent. No explanation can be given for these exceptional cases. Fortunately, they seem to become less frequent with increasing experience.

The results of a rather extensive application of the technique here described are presented in another paper in this issue.

We make grateful acknowledgment of the kindness of the late Professor R. A. Gortner of the University of Minnesota in supplying us with samples of purified melanin, and for valuable information; to our colleague, Dr. D. L. Fox for the use of the spectrophotometer under his charge as well as for advice on various matters, and to Mr. Sheldon Crane for assistance in the photometric work.

SUMMARY

A method is described of preparing a solution (transparent colloidal suspension) of the melanin of fishes, and of assying this colorimetrically. The method includes hydrolysis of the tissues in a boiling alkaline solution, precipitation, centrifuging, further hydrolysis in acid and removal of acid and digestion products by dialysis; finally dissolving the melanin in dilute NaOH. Readings of the various samples were made with a tint-photometer, but a spectrophotometer could have been employed, perhaps, to advantage.

For the interpretation of these readings, curves were drawn, based upon solutions of purified melanin of known concentration.

The transmission "curve" of the various wave-lengths through such a melanin solution was likewise obtained by the use of a spectrophotometer. This proved to be a nearly straight line from a low point in the blue to a high point in the red.

With lapse of time, alkaline "solutions" of melanin rather rapidly deteriorate, even in the dark, becoming increasingly transparent to all wave-lengths, but proportionately more to the red than would result from mere dilution.

LITERATURE CITED

DAWES, B., 1941. The melanin content of the skin of Rana temporaria [etc.]. *Jour. Exper. Biol.*, **18**: 26–49.

GORTNER, R. A., 1910. Studies of melanin. *Jour. Biol. Chem.*, **8**: 341–363.

GORTNER, R. A., 1911. On melanin. *Biochem. Bull.*, **1**: 207–215.

HEINLEIN, H., 1924. Zur Kenntnis melanotischer Pigmente. *Biochem. Zeitschr.*, **154**: 24–34.

KUDO, T., 1922. Veränderung der Melaninmenge beim Farbwechsel der Fische [etc.]. *Arch. f. Entwick. d. Org.*, **50**: 309–325.

SALKOWSKI, E., 1920. Ueber die Darstellung und einige Eigenschaften des pathologischen Melanins. *Virchow's Arch.*, **227**: 121–137.

SUMNER, F. B., 1940a. Further experiments on the relations between optic stimuli and the increase or decrease of pigment in fishes. *Jour. Exper. Zool.*, **83**: 327–343.

SUMNER, F. B., 1940b. Quantitative changes in pigmentation, resulting from visual stimuli in fishes and amphibia. *Biol. Rev.*, **15**: 351–375.

SUMNER, F. B., AND P. DOUDOROFF, 1937. Some quantitative relations between visual stimuli and the production or destruction of melanin in fishes. *Proc. Nat. Acad. Sci.*, **23**: 211–219.

SUMNER, F. B., AND P. DOUDOROFF, 1938. Some effects of light intensity and shade of background upon the melanin content of Gambusia. *Proc. Nat. Acad. Sci.*, **24**: 459–463.

SUMNER, F. B., AND D. L. FOX, 1933. A study of variations in the amount of yellow pigment (xanthophyll) in certain fishes [etc.]. *Jour. Exper. Zool.*, **66**: 263–301.

SUMNER, F. B., AND D. L. FOX, 1935. Studies of carotenoid pigments in fishes. II. Investigations of the effects of colored backgrounds [etc.]. *Jour. Exper. Zool.*, **71**: 101–123.

VILTER, V., 1931. Modifications du système mélanique chez les axolotls [etc.]. *C. r. Soc. de biol.*, **108**: 774–777.

Contribution No. 195

Reprinted from Biological Bulletin, Vol. 84, No. 2, 195–205, April, 1943
Printed in U. S. A.

A FURTHER REPORT UPON THE EFFECTS OF THE VISUAL ENVIRONMENT ON THE MELANIN CONTENT OF FISHES[1]

F. B. SUMNER

(*Scripps Institution of Oceanography of the University of California, La Jolla*)

Introduction

That a sufficiently long sojourn upon a black or white background may result in a marked increase or decrease in the pigmentation of fishes and amphibia has been shown by several investigators (Cf. recent review by the present author, 1940*b*). Sumner and Wells (1933) found pronounced changes of this sort in *Lebistes reticulatus*, in which not only the number of melanophores per unit area of skin was found to be markedly affected, but also the amount of melanin per cell. These changes were portrayed unmistakably in our published photographs, but no attempt was made at that time to measure their extent.

For two other fishes, *Gillichthys mirabilis* and *Gambusia affinis*, Sumner and Doudoroff (1937, 1938) made quantitative determinations of the amount of melanin present in animals of different experimental history. These determinations were beset with considerable obstacles which seriously affected their precision. Certain conclusions were, however, established with reasonable certainty in both of these series of experiments. (1) Fishes of "black" history yielded considerably more melanin than fishes of "white" history, while those from intermediate backgrounds (grays) were intermediate in this respect. (2) Within broad limits, the intensity of the incident light was a minor factor in the production of such differences.

A more definite quantitative relation was indicated as possible between the albedo[2] of the background and the amount of melanin produced (or retained). In Figure 3 (Sumner and Doudoroff, 1937) and Figure 1 (Sumner and Doudoroff, 1938), it is evident that the relation between these two variables is not a linear one. If the figures for albedo stated on page 213 (1937) be converted into percentages of "white" (the latter being regarded as 100) and if 0.74 be taken as the value for "black," the Gillichthys values of Table I, "A" (means), form a nearly perfect logarithmic series.[3] This relation does not hold at all well for the "B" fishes, however, though the arrangement here is likewise of the "hollow curve" type.

The relations shown by the "A" set of these fishes suggested as possible the rule that the amount of pigment produced (or retained) varied inversely as the logarithm of the albedo of the background. The analogy between such a formulation and the so-called "Weber-Fechner Law" in human sense-physiology was at once recognized.

---

[1] Contributions from the Scripps Institution of Oceanography, New Series, No. 195.

[2] By *albedo* is meant the specific reflectivity of a given surface, i.e., the proportion of incident light which that surface reflects.

[3] This mode of treating the data is not in the least arbitrary. Our original attempt to state the albedo of our "white" in absolute terms was not successful and has been abandoned. But the figures for "albedo," referred to "white" as a standard, are fairly exact. The figure of 0.74 for "black" is taken from Sumner (1940a). While only approximate, it is certainly a fairer figure than 0.

Because of the difficulties encountered in our earlier attempts to assay melanin, the present author next resorted to counting melanophores in definite areas of the skin of *Lebistes reticulatus* (Sumner, 1939, 1940*a*). Accurate counting was rendered possible by causing the pigment-masses of the melanophores ("melanosomes") to concentrate through the action of adrenalin.

In these experiments, the previously observed gradient in the effects of the various backgrounds, from black to white, was clearly manifest. And again, the logarithmic relation between stimulus and pigmentation was plainly indicated, though the values for the "black" fishes were somewhat too low (1940*a*, Figures 3 and 4). Once more, any differences due to the intensity of general illumination, if significant at all, were slight in comparison with those resulting from the shade of the background.

While there could be no doubt that the melanin content of black-adapted fishes was greater than that of white-adapted ones, it was plain that more exact quantitative expressions for these differences in melanin content were desirable.

Further endeavors to perfect a method of assaying melanin were resumed by Sumner and Doudoroff, who have discussed the outcome of their completed task in this issue of the *Biological Bulletin* (pp. 187–194). The present paper reports the results of two series of experiments in which this method has been applied successively to two species of fish, *Fundulus parvipinnis* and *Girella nigricans*. Since, of these two, the results from the latter species were of far greater significance, they will receive the principal attention.

I must here acknowledge the valuable assistance of Dr. Peter Doudoroff and Mr. Carl I. Johnson in the preparation of some of the equipment here employed, and of Mr. Urless Lanham and Mrs. J. F. Wohnus in connection with the care of the fishes and some of the subsequent laboratory procedure. Acknowledgment is also due Messrs. P. S. Barnhart and C. W. Palmer for obtaining the supply of fishes.

METHODS

In the case of Girella, eight to ten of the fishes, averaging (two months later) about 60 mm. in length, were placed in each of the 24 experimental bowls. Running sea-water was supplied to these. The fishes were fed three times a week, chiefly with beach-worms (Thoracophelia) and canned shrimps. Debris (feces and food remains) was removed from the bottoms of the bowls by siphoning, nearly every day, and the bowls were scoured once a week to remove bacterial and algal growths.

The bowls were kept in six cabinets, painted white within and lighted by electric lamps overhead. Four bowls of the same albedo were kept in each of five cabinets, lighted by a 100-watt lamp. A second set of four black bowls was kept in the sixth cabinet, lighted by a 10-watt lamp. The cabinets were all kept lighted night and day.

In the case of Fundulus, only five cabinets were employed, all with 100-watt lights, and the extra set of black bowls was omitted.

The intensity of illumination in the more strongly lighted cabinets, as determined with a Weston Photronic Cell placed at the customary water-level in the bowls, the latter being covered by a ¼-inch screen, was about 33 foot-candles. In the less brightly lighted cabinet, it was about 2 foot-candles.

The bowls were of clear, uncolored glass, with nearly straight sides, 24 cm. in diameter and 15 to 17 cm. deep. As has been our practice, these bowls were painted on the outside with several coats of auto enamel. Black and white enamel were used, singly and in mixtures which provided the three shades of gray. In order to eliminate bright gleams, from surface reflection, the bowls were given a frosted surface by sand-blasting on the inside. This surface becomes transparent when covered with water.

Albedos were determined by a method described more fully earlier (Sumner, 1940*a*). Light reflected from the bottoms was measured by the use of a photronic cell and galvanometer. The readings thus obtained did not reveal the absolute albedos of the surfaces in question (i.e., ratios of reflected to incident light), since it was impracticable with available equipment to measure the incident light reaching the bottom of a bowl nearly filled with water. This was not necessary, however, because the important thing to know in these experiments was the relative reflectivity of these various surfaces, as compared with one another. As already stated, these figures were reduced for present purposes to a common standard, the reflectivity of the white bowls being taken as 100.

Since, as already indicated, the bottoms of the bowls were unavoidably befouled part of the time, another set of albedo readings was taken with the bowls in this state. For this purpose, an attempt was made to reproduce their average condition preceding the—usually daily—siphoning off of the debris. The result was necessarily only a rough approximation. Table I gives the figures for each type of bowl, with and without the debris. The figures in the third column, which represent an average of the clean and the dirty conditions of the bowls, are the ones here employed in considering the relations between albedo and pigmentation.[4]

TABLE I

Albedos of the various backgrounds, relative to white as a standard

	Without debris	With debris	Approximate average condition
White	100.00	100.00	100.00
Pale gray	38.17	37.31	37.74
Medium gray	17.84	17.77	17.80
Dark gray	6.56	7.22	6.90
Black	0.94	1.94	1.42

The present table gives the albedos of the bowls used for Girella. The set used for Fundulus received the same shades of paint (many of the bowls were identical), with the exception of the "dark gray" bowls, which were unintentionally made considerably paler, having an albedo of 11.79, instead of 6.90.

---

[4] In Table I of Sumner, 1940a, the figures in the column headed "With Debris" are the ones employed in the text discussions of that paper, but those figures are based upon a less extreme condition of fouling than that dealt with in the present paper.

In the Girella experiments, about half of the fishes were removed for the melanin assay two months (59 to 61 days) after the commencement of the experiment, the remainder being removed two months later (a total of 122 to 123 days). Four samples were taken from each type of background on each of these occasions. Each sample consisted of from three to five fishes. Two series of Fundulus were likewise assayed, after periods of 24 to 26 days and 57 to 59 days, respectively. Here the four samples from each background consisted of five to seven fishes.

The treatment to which this material was subjected has been described by Sumner and Doudoroff in the present number of the *Biological Bulletin* (pp. 187–194).

RESULTS

For Girella, notes were made on the appearance of the living fishes near the close of each period of the experiment.

(1) Differences of shade, resulting from the influence of the backgrounds, were very marked. Most of the fishes in the black bowls were so black as to be nearly invisible against these backgrounds. Those in the white bowls were very pale, appearing almost white (for a few seconds only) when transferred to a black bowl. Fishes in the intermediate bowls were correspondingly graded.

(2) Except in the black bowls, and to a less degree in the dark gray ones, however, the adaptation was far from complete, even after four months. The white-adapted fishes, while very pale, appeared far from white, *on their own backgrounds*, and could not even be regarded as well concealed. The fishes on pale and medium gray were also considerably darker than their respective backgrounds.

TABLE II

Melanin content (mg./gm.) of Girella killed after two months

Black (10W)	Black (100W)	Dark gray	Medium gray	Pale gray	White
1.35	[0.91]	0.86	0.78	0.63	0.81
1.40	1.37	0.95	0.82	0.68	0.82
1.60	1.52	1.03	0.93	0.80	0.89
1.61	1.55	1.21	—	—	0.93
1.49	1.48	1.01	0.84	0.70	0.86

TABLE III

Girella killed after four months

Black (10W)	Black (100W)	Dark gray	Medium gray	Pale gray	White
1.33	1.22	0.88	0.63	0.50	0.61
1.51	1.40	1.02	0.71	0.57	0.62
1.51	1.55	1.12	0.73	0.61	0.65
1.58	1.57	1.22	0.91	0.64	0.79
1.48	1.43	1.06	0.74	0.58	0.67

(3) Great individual differences of shade were sometimes manifested among fishes in the same bowl, particularly in the medium and dark gray bowls. Among the latter it was noted that the two extremes could even be characterized as "pale" and "very dark," relative to their background. Whether or not such visible differences resulted from actual differences in the amount of melanin was not determined. Apparently, they were more or less permanent for the individuals concerned.

(4) The greater part of the induced differences of shade, even after four months, was of the transitory ("physiological") type. Strikingly rapid changes were still visible, following the transfer of a fish from one background to another. Again, the effect of the chloroform vapor with which the fishes were killed was to level down these differences, the pale fishes becoming much darker and vice-versa. While no figures are available for the color differences of the living fishes, it is certain that the difference between those of "black" and "white" history was many times the maximum difference ($2\frac{1}{2}$: 1) shown by the melanin extracted from these same lots.

Figures for the melanin content of the skins of the various lots of Girella are shown in Tables II and III,[5] and some of the relations among these values are depicted graphically in Figures 1 and 2. Each of the values comprised in the tables is derived from one of the "samples" of three to five fishes referred to above, and in most cases it represents the mean of two sub-samples, into which the dissolved material was early divided. The figures in each column are arranged in order of magnitude, without reference to the chronological order of the analyses.

The range of magnitude displayed in each of the columns of these tables is plainly considerable. This despite the fact that each of these figures represents the mean condition of several individuals. Considering the very great individual variability of some fishes in respect to pigmentation, however, these differences among the melanin values for small samples are not surprising (Sumner and Doudoroff, this issue, p. 192).

It seems probable that the figure in brackets in Table II (0.91) is due to some accident or error of procedure, though no such error was perceived at the time. This low value is not approached by any other of the 16 figures comprised in the four columns for "black" fishes in the two tables. It will also be noted that the mean for the column containing it (black, 100W.), when the aberrant figure is omitted, agrees rather closely with the other three means for "black." If this (possibly correct) figure is included, the mean for its column is 1.34.

The two vacant spaces in Table II result from the accidental loss of the corresponding samples of material.

Aside from one feature, the curves (Figure 1) show much the same form as those presented in previous papers (Sumner and Doudoroff, 1937, 1938; Sumner, 1940, *a* and *b*). From black to pale gray, the arrangement of the values approaches very closely a logarithmic one, at least for the 4-month series (Figure 2).

In one respect, however, the curves based upon these experiments differ markedly from any of those shown in our earlier papers. In the present case, there is an actual rise from pale gray to white, the reality of this relation being emphasized by the fact that it is shown in both the 2-month and the 4-month series. Here we have an abrupt departure from the logarithmic relation. The possible significance of this will be discussed later.

---

[5] These melanin values were obtained by reference to curves based upon "standard" solutions of melanin derived from silky fowls (see preceding paper, p. 190). It can hardly be assumed that they represent with any exactness the absolute quantities of melanin in our fishes.

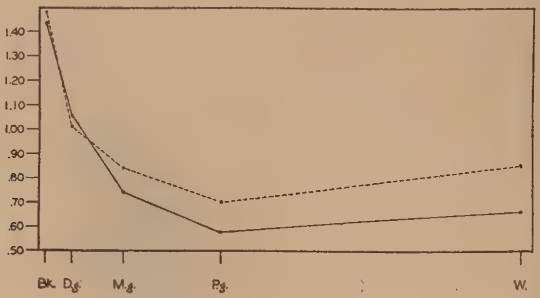


FIGURE 1. Melanin content (milligrams per gram of original weight of the fishes) of Girella, after sojourn on various backgrounds; broken line after two months, continuous line after four months. Abscissas = albedos; ordinates = melanin.

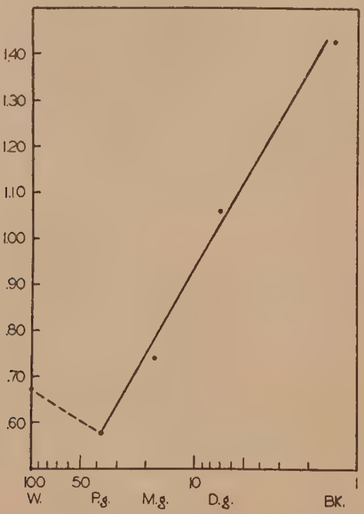


FIGURE 2. Melanin content of the 4-month series plotted against the logarithms of the albedos of the backgrounds.

Comparison of the 2-month and the 4-month series brings out certain points of interest. In respect to the values for black and dark gray, the two series differ but slightly from one another. Beyond the latter point, however, the two curves diverge steadily, that for the 4-month fishes falling well below that for the 2-month ones. This is not surprising when we consider that freshly caught fishes, when subjected to these artificial conditions, commonly lose pigment freely upon the paler backgrounds, but undergo little increase upon the darker ones.[6] This loss, as might have been expected, has progressed considerably further in four months than in two. It is not impossible, indeed, that longer subjection to the experimental conditions would have resulted in depressing the value for "white" below that for "pale gray."

To consider these differences quantitatively, we find that after two months the melanin yield of the "black" fishes was about 1¾ times that of the "white" ones, and more than twice as great as that of the "pale gray" ones. For the 4-month series, the figures are 2 + and 2½, respectively. As already stated, these differences are slight in comparison with the very great differences in the appearance of the living fishes.

Fundulus, as already stated, yielded far less instructive results than Girella. Earlier experiments had shown that visible responses to backgrounds were much less pronounced in the former than in the latter. Indeed, the living "black" fishes were far from black and did not seem to darken further after the first few days.

Mean melanin values (mg./gm.) for the 2-month period are as follows:

Black	0.37
Dark gray	0.40
Medium gray	0.36
Pale gray	0.34
White	0.26

These figures are based upon four samples (five to seven fishes each) in the case of the three grays, only three samples in the case of white and black. The significance of any of these differences may well be doubted, with the exception of that between "white" and the other four members of the series. In general, the variability among the figures for each background is high.

Comparing the figures for Fundulus with those for Girella, it is plain that the former, in all cases, is a much less highly pigmented fish than the latter. The ratio, indeed, is nearly 1 : 4. Again, the influence of the background is much less pronounced in Fundulus than in Girella. In the former it has been seen that there is little or no difference in melanin content between the fishes kept on black and those kept on the various shades of gray, while the "white" fishes seem to have lost only 30 per cent of their melanin.

Discussion

Despite the marked differences shown by the different fishes in our present and similar previous experiments, certain common features are obvious in their reactions to the background. In every case, among these various fishes (Gillichthys, Gambusia, Lebistes, Girella, Fundulus) quantitative changes have been brought about in the amount of melanin contained in the skin. In all of these, except Fundulus, the greatest melanin content was shown by the black-adapted fishes, and in all except Girella the lowest melanin content was shown by the white-adapted fishes. For the most part, too, the intermediate values for this pigment were graded in the order of decreasing albedo of the background. Exceptions to this were the position of "white" in the "B" series (but not the "A") in Gillichthys, the transposed relation between "dark gray" and "medium gray" in Gambusia, the position of "white" in Girella and the position of "black" in Fundulus. It should be added, however, that in no case did one of these values deviate more than one place from its expected position in the series.

---

[6] However, fishes which have become depigmented as a result of subjection to pale backgrounds will regain this pigment when transferred to black (Sumner and Wells, 1933).

In none of these fishes were the melanin values, when plotted against albedo, arranged in linear fashion. In every case except Fundulus, they were so arranged as to form a "hollow" curve. (Since Fundulus displayed such limited pigmental reactions, it may be omitted from further discussion of this subject.)

In our most extensive experiments—those upon Gillichthys, Lebistes and Girella—the whole or greater part of this curve was of the logarithmic type. In series "A" of the Gillichthys experiments this arrangement was nearly perfect. In the Lebistes experiments it held fairly well except for "black," the value for which was somewhat too low. In the Girella experiments—the most extensive of all—it held very closely except for "white," the value for which was too high, so much so as to throw it out of proper alignment in the series (Figures 1 and 2).

We have more than once pointed out the possible analogy between the relations shown in these pigmental responses of fishes to albedo and the phenomena of human sense physiology which have been generalized as the "Weber-Fechner Law." Throughout a considerable range of stimuli, it has been found that equal increments of sensation (just perceptible differences) result from *proportional* rather than equal increments of the stimulus. In Fechner's well-known formulation, Sensation = C log Stimulus.

It is commonly recognized that this is a generalization of limited application, and that at best it holds for a limited range of stimuli. Particularly is it known that the relation in question breaks down at low intensities of the stimulus, and sometimes also at high intensities (Woodworth, 1938, pp. 430 *et seq.*). Whether or not the low melanin value for "black" in the Lebistes experiments and the high value for "white" (i.e., lessened depigmentation) in the Girella experiments could be regarded as examples of the "breakdown" of any such general rule at low and high intensities of stimulation is, of course, quite questionable. For the present, we can say only that the analogy between these phenomena of pigmentary response and the phenomena comprised under the "Weber-Fechner Law," however suggestive this may be, is perhaps an entirely superficial one.

That the intensity of incident light, within wide limits, is much less effective than albedo in causing differences in pigmentation, has already been insisted upon. It is possible, however, that different species differ from one another in the degree to which light intensity is effective in this matter. In the Gillichthys experiments, it was found that 7 per cent more pigment was formed in the cabinet which was lighted by two 200-watt lamps than in the cabinet which was lighted by two 10-watt lamps. In the Lebistes experiments, likewise, a small but inconstant difference was shown, fishes from the more highly lighted cabinet (64(±) foot-candles) averaging slightly higher in melanin content than those from the less highly lighted one (0.24 f.c.). On the other hand, the rather unsatisfactory experiments with Gambusia and the recent far more thorough experiments with Girella furnish no evidence that in these fishes the wide differences of illumination resulted in any pigmental differences.[7] In the former the illuminations ranged, approximately, from 90 to 0.25 foot-candles; in the latter they were 33 and 2 f.c. respectively. It cannot be stated positively, however, that all these differences in results are due to differences of species. Several other factors (time and temperature, as well as light) varied from one experiment to another.

In respect to the amount of pigmental change brought about by these differences of albedo, we find once more that the various species employed differed rather widely from one another. Here again, we must bear in mind the reservation expressed in the preceding paragraph, although specific differences have certainly played an important role.

In counts of the melanophores in Lebistes (Sumner, 1940*a*), the mean number of melanophores in a definite area of skin was 2½ times as great in the black-adapted fishes as in the white-adapted ones. In some lots of the same species reared by Sumner and Wells (1933), however, the differences, while not determined quantitatively, appear from photographs to have been far greater than this. In the present Girella series, we may repeat, the differences in melanin content were 2 and 2½ : 1. In the Gillichthys experiments of Sumner and Doudoroff (1937), on the other hand, the melanin content of the "black" fishes, after 87 days, exceeded that of the "white" ones by only about 30 per cent. Finally, in the recent Fundulus experiments, there were no certain differences among any of the first four albedos (black to pale gray), while the average of these exceeded the "white" value by about 42 per cent (of the latter).[8]

Before concluding, we may dispose of one possible serious criticism of our experimental procedure. It is needless to say that, when a number of fishes are kept together in bowls of the size here used, a not inconsiderable part of the visual field of every fish is occupied by its neighbors. It may be asked, accordingly, whether this circumstance may not be sufficient to invalidate any conclusions based upon the albedos of the bowls themselves.

Against any such possible objection there are rather strong arguments. It was long ago shown for flatfishes by the present writer (Sumner, 1911) that the chromatic changes of fishes are far more influenced by stimuli received from the bottom of a container than from its lateral surfaces. That this type of response is not restricted to bottom-dwelling fishes was amply shown by N. A. Wells and myself for *Fundulus parvipinnis* in 1930. In these experiments (results unpublished), two sets (four each) of 5 × 7-inch battery-jars were painted, one with the bottoms black and the walls white, the other in the reverse condition. Fishes (four in each jar) were kept for 22 days under these conditions, and were compared several times during this period in all-white jars, the two contrasted lots being poured into the latter simultaneously. With probably not a single exception, all of the fishes from the white-bottomed jars were paler than any of those from the black-bottomed ones. This despite the fact that the area of the walls of the jars, below the water line, exceeded that of the bottoms in a ratio of more than 3 : 1.

---

[7] That is, if the bracketed figure in Table II is left out of consideration. If included, it would change the mean figure in the "wrong" direction, i.e., give a *lower* value for the more highly lighted fishes.

[8] Dawes (1941) reports a difference of 60 per cent in skin melanin between black-adapted and white-adapted frogs, after about five weeks. He believes that a greater change from their original condition occurred in his black-adapted than in his white-adapted specimens, since the former depart more from "the mean animal which was not kept on any particular background" (!). Dawes's claim for complete priority in the demonstration of such changes can hardly be taken seriously in view of the facts reported in the preceding pages.

In our various experiments with painted aquaria, one fish's neighbors commonly formed part of its lateral field of vision; much less commonly did they obscure its view of the bottom or any considerable part of this. Moreover, as adaptation to the background proceeded, the biological portion of this background came to contrast less and less with the remainder.

Summary

The results are presented of recent experiments upon *Girella nigricans* and *Fundulus parvipinnis*, together with a comparative discussion of previous similar experiments by the author and collaborators.

In this latest series, Girella was exposed for two and for four months to backgrounds of five albedos (black, three grays and white), lighted by 100-watt electric lamps; while another set was exposed to black backgrounds only, lighted by a 10-watt lamp. In the Fundulus series, only the brighter lights were employed.

The effects of this treatment upon melanin production (or loss) were much more pronounced in Girella than in Fundulus. In Girella, the amount of melanin in the skin, after four months, was 2½ times as great in fishes from the black containers as in those from the pale gray containers. This difference in melanin content was trivial, however, in comparison with the difference in appearance of the living fishes. The latter was due, for the most part, to the transitory disposition of the pigment within the chromatophores.

The minimum melanin content in Girella was not obtained from the occupants of the white containers, but from those of the pale gray containers. The four values from "black" to "pale gray" formed, however, a descending series having a distinctly logarithmic arrangement.

In Fundulus no such arrangement was found, there being only one significant difference, that between fishes of "white" history and all of the others. The mean value of the latter is less than 1½ times that of the former.

The data from this and similar previous experiments show that in four of the five species studied, the melanin values, when plotted against albedo, form "hollow" curves, and that this arrangement, in some of the cases, is definitely logarithmic. The possible analogy between this tendency and the "Weber-Fechner Law" is discussed.

Fishes kept in black and dark gray bowls showed little change after two months. Those from the other bowls, most of all from the white, showed considerable further decreases between the 2-month and the 4-month periods. It is probable that the differences between the dark-adapted and pale-adapted fishes resulted less from increase of pigment in the former than from decrease in the latter.

No probable difference existed between black-adapted fishes kept under 100-watt lights and the same when kept under 10-watt lights, the lighting here being in a ratio of about 16 : 1.

Reasons are given for believing that the chromatic response of a fish to its background is not much interfered with by the presence of other fishes in the same container.

LITERATURE CITED

Dawes, B., 1941. The melanin content of the skin of Rana temporaria [etc.]. *Jour. Exper. Biol.*, **18**: 26–49.

Sumner, F. B., 1939. Quantitative effects of visual stimuli upon pigmentation. *Amer. Nat.*, **73**: 219–234.

Sumner, F. B., 1940a. Further experiments on the relations between optic stimuli and the increase or decrease of pigment in fishes. *Jour. Exper. Zool.*, **83**: 327–343.

Sumner, F. B., 1940b. Quantitative changes in pigmentation, resulting from visual stimuli in fishes and amphibia. *Biol. Rev.*, **15**: 351–375.

Sumner and Doudoroff, 1937. Some quantitative relations between visual stimuli and the production or destruction of melanin in fishes. *Proc. Nat. Acad. Sci.*, **23**: 211–219.

Sumner and Doudoroff, 1938. Some effects of light intensity and shade of background upon the melanin content of Gambusia. *Proc. Nat. Acad. Sci.*, **24**: 459–463.

Sumner and Wells, 1933. The effects of optic stimuli upon the formation and destruction of melanin pigment in fishes. *Jour. Exp. Zool.*, **64**: 377–403.

Woodworth, R. S., 1938. Experimental Psychology. Henry Holt, New York, 889 pp.

Contribution No. 196

Copyright, 1943, by the Society for Experimental Biology and Medicine.
Reprinted from PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE,
1943, **52**, 133-134.

14058 P

Bacterial Oxidation of Hydrocarbons in Marine Sediments.*

G. DAVID NOVELLI. (Introduced by Claude E. ZoBell.)
From the Scripps Institution of Oceanography, University of California, La Jolla.

It has been demonstrated[1] that certain bacteria, widely distributed in nature, are capable of utilizing petroleum hydrocarbons as a source of energy. Recently Grant and ZoBell[2] have demonstrated the presence of such bacteria in marine environments.

If such bacteria are active in marine sediments, *in situ,* they may account for the dearth of hydrocarbons[3] in this material; thereby influencing the present theories of petroleum genesis. Therefore studies were undertaken to obtain more information on factors which may influence the activity of these bacteria in potential source-sediments.

Experimental. The sediments used in this investigation were samples of bottom mud from Mission Bay and from the Channel Island region. To demonstrate the presence of hydrocarbon-oxidizing bacteria in these materials, small amounts of the sediment were added to tubes containing 75% sea water, 0.1% NH_4Cl and a large drop of sterile paraffin oil. Uniform turbidity in the tubes or a pellicle of growth at the oil-water interface after 7 to 9 days incubation indicated the presence of these organisms. These cultures were purified by repeated transfers and by plating procedures. Using this technic, cultures able to oxidize a variety of hydrocarbons have been isolated from most of the sediments tested.

To test hydrocarbon-oxidation in the sediments, the muds were mixed with sterile paraffin oil, or in some cases a lubricating oil, and were added to sterile bottles. Oxidation of the hydrocarbon was determined by the extent of oxygen consumption which was measured manometrically.

Table I shows the results of a typical experiment in which 50 g of sediment from Mission Bay were used. The test cultures contained 2 ml of the particular oil. The controls represent biological oxidation of the organic matter in the muds. It is evident that considerable O_2 was consumed in the presence of the hydrocarbon. The quantity of hydrocarbon oxidized was calculated on the assumption that 3.5 mg of O_2 are necessary to completely oxidize 1 mg of a hydrocarbon. The O_2 consumed in 9 days was approximately ¾ the total capacity of the bottles, indicating that hydrocarbons may be oxidized in sediments stored in containers if sufficient oxygen is present. Even if suitable examination of marine sediments do not reveal the presence of hydrocarbons it does not preclude the possibility of their

TABLE I.
Milliliters of Oxygen Consumed by Bacteria in Mud After Different Periods of Time at 25°C. The Amount of Hydrocarbon Oxidized Is Also Given.

	Incubation period in days 3	5	7	9	Hydrocarbon oxidized after 9 days
	ml	ml	ml	ml	mg
1. Mud only	0.5	1.5	2.7	4.5	
2. ” ”	1.3	3.3	3.9	5.4	
3. ” + paraffin oil	3.4	8.9	12.6	16.3	4.82
4. ” + ” ”	1.3	6.5	11.7	15.3	4.17
5. ” + lubricating oil	3.5	11.1	18.4	22.1	6.88
6. ” + ” ”	2.2	7.4	14.3	20.4	6.20

---

* Contributions from the Scripps Institution of Oceanography, New Series, No. 196, and paper No. 3 from Project 43A sponsored by the American Petroleum Institute.

[1] Bushnell, L. D., and Haas, H. F., *J. Bact.*, 1941, **41**, 653.

[2] Grant, C. W., and ZoBell, C. E., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 266.

[3] Trask, P. D., *Problems of Petroleum Geology*, Am. Assn. Petrol. Geologists, Tulsa, Okla., 1934.

having been formed, but subsequently oxidized by bacteria, *in situ*.

It was noticed in the course of these experiments that the amount of water present in the sediments seemed to affect the oxygen consumption. To test this observation an experiment similar to that outlined in Table I was set up. Controls and tests to which 25 ml of sterile 75% sea water was added were included. The addition of water did not seem to affect the oxidation of the hydrocarbon since the relative amounts oxidized in the tests with and without added water were the same. The controls without water consumed much more oxygen than did those with added water. Thus it would seem that hydrocarbons in marine sediments are oxidized preferentially to organic matter.

Contribution No. 197

Made in United States of America

Reprinted from THE QUARTERLY REVIEW OF BIOLOGY
Vol. 18, No. 2, June, 1943, pp. 154–164

SEXUAL DIFFERENTIATION IN MOLLUSKS

I. PELECYPODS

BY W. R. COE

Osborn Zoological Laboratory, Yale University, and Scripps Institution of Oceanography, University of California

(Contributions from the Scripps Institution of Oceanography, New Series, No. 197)

AMONG the more than 10,000 species representing the class Pelecypoda may be found every grade of sexual differentiation, from species that are strictly of separate sexes to those that are almost invariably functionally hermaphroditic. Even two species belonging to the same genus may differ considerably in sexuality. And in a relatively few species the functional sexual phase of some or of all the individuals may change during the animal's lifetime. Indeed there are some species in which nearly every normal individual experiences several alternating male and female sexual phases. Hermaphroditism may likewise be complete, partial or occasional.

Because of these wide variations in the expression of sexuality, not only in different species but in different individuals of the same species and at different periods of life, the bivalve mollusks promise to offer evidence of some importance toward an understanding of the general problem of sexuality in organisms. This evidence must be examined as to its bearing on embryology, physiology and genetics.

Except in those species that are strictly of separate sexes (unisexual, or dioecious) the gonad contains the antecedent cells of both eggs and sperm. As a general rule the male cells mature in advance of the female cells, whereby the initial phase of functional sexuality is male. Such individuals are said to be protandric. Less frequently the initial phase is female and the individual is proterogynous. In functional hermaphrodites the eggs and sperm develop concurrently.

When each normal individual experiences two or more alternative sexual phases it is quite illogical to treat the sperm-producing and egg-producing stages as males or females as if they were distinct categories of individuals. They are merely temporary phases in the life of the same individual. Furthermore the proportions of individuals in each of these phases after the first would seem to have less significance than some investigators have supposed, since it is obvious that if twice as many individuals are found in one of these phases as in the other, that may be merely an implication that one of the phases lasts twice as long as the other. It might also mean that some are older than others.

The sexual conditions in the pelecypods are sometimes further complicated by the occurrence of neoteny, or juvenile sexuality. In such cases the individual may form a small number of gametes when it is very young and only a small fraction of its definitive size. This juvenile phase may may be more or less aborted and, as mentioned on the following pages for *Venus* and *Ostrea* may be of a different sexual type than that found in the adult. Consequently the sexuality of a species can be determined only by the examination of numerous individuals at all periods of life.

At the outset it must be recognized that the process of changing from one sexual phase to another is usually, but not invariably, gradual, with an intervening phase during which the gonad presents all possible grades of hermaphroditism, or ambisexuality. Since in many species it is impossible to examine the gonad of any one individual at successive stages of its life, it becomes necessary to infer its history from an examination of other individuals at different ages. These individuals, if protandric, might be erroneously thought to be males if examined when young, females when older, and hermaphrodites during the stage of sexual transformation. They represent, on the contrary, merely the sexual phases experienced by each normal individual during its lifetime.

In some species, however, definite proof of the umber of changes of sexual phase experienced by ny individual can be obtained by marking the hell and observing the sexuality at succeeding pawning periods. This is likewise feasible with astropods. But with other forms, as the woodoring bivalves, that is not possible and recourse must be had to observations of the gonads of many ifferent individuals of known ages. In the arviparous species the presence of embryos or arvae in the gill chambers or mantle cavities is ufficient evidence as to the character of the preeding sexual phase.

With these facts in mind a brief survey of the exuality of the whole group of bivalves may be ndertaken. For more than half the total number f described species the sexual conditions are quite nknown or undescribed. For the others, approximately 96 per cent are reported as of separate exes or that type of sexuality is inferred. Less han 400 of the 10,000 described species are known o deviate from a strictly dioecious, or unisexual, ondition, except for an occasional case of hermaphroditism due to an abnormality of development. Of those species that are normally monoeious (hermaphroditic, bisexual, ambisexual) some re uniformly functionally hermaphroditic, producing eggs and sperm at the same time, while others how various grades of mixed sexuality or changes in sexuality at some period of life.

The pelecypods have no copulatory organs or ther external sexual characteristics, with the xception that in certain dioecious species of *Unio*, *Astarte* and a few other genera the two sexes, when adult, can be distinguished by the shape of he shell. Otherwise it is necessary to examine he gonad unless it is possible to observe the ndividual during the act of spawning. Accesory sexual organs are absent with the exception f the seminal vesicles, found only in *Xylophaga* nd *Cuspidaria*, and the brood chambers on the ills of various larviparous species.

DEVELOPMENT OF PRIMARY GONADS

The sexuality of any individual obviously depends primarily upon its hereditary characterstics and these first find expression in the gonads. Consequently the history of the gonads must be ollowed in order to obtain reliable information elative to the prospective sexuality of any individual.

The primary gonads arise from a pair of germinal primordia situated in the posterior portion of the body, ventral to the pericardium and in close proximity to the visceral ganglia and the renal organs. Each of the primordia develops into a profusely branching system of follicles surrounding the intestine in the mesosoma, or visceral mass. In the mussels the gonads extend also into the mantle lining both valves of the shell and in *Anomia* into the mantle on the right side of the body.

The constituent cells of each follicle soon become differentiated into nutritive follicle cells and primary gonia. If the individual is to become hermaphroditic the primary gonia soon give rise to cells of two types, clearly distinguishable as spermatocytes and ovocytes. But even in dioecious species there may be indications of ambisexuality in the primary gonad and some ovocytes are often formed in the early spermary. Such ovocytes undergo cytolysis during spermatogenesis

A. AMBISEXUALITY: MONOECISM

For convenience of reference the various grades of ambisexuality may be considered as consisting of the four following categories: (1) functional ambisexuality (functional hermaphroditism), (2) consecutive sexuality, (3) rhythmical consecutive sexuality, and (4) alternative sexuality. Since the terms "bisexual" and "bisexuality" as currently used by different writers may indicate either a monoecious condition of an individual or a dioecious condition of a species they may well be avoided. The term "ambisexual," if used strictly with reference to individuals having gonads composed of both male and female sexual cells, is less ambiguous than either bisexual or hermaphroditic.

1. *Functional ambisexuality* (*Functional hermaphroditism*)

Functional hermaphroditism is found in nearly all phyla of invertebrates and in every phylum of plants. There is here no incompatibility between the male and female sex organs and both types of sexual cells may be produced simultaneously. Not infrequently, however, this condition is preceded by a brief period of maleness or occasionally of femaleness. In some of these species the extent of protandry is increased by unfavorable conditions and can be controlled experimentally.

(*a*) *Normal.* This implies a strictly monoecious, or ambisexual, condition, with the con-current production of gametes of both sexual types by each individual. In some species the male and female elements are in separate gonads, with separate efferent ducts; in others there are male and female portions of a single gonad, while still others have both spermatogenic and ovogenic cells in the same follicle. A tendency toward protandry is general but some individuals discharge both types of gametes at the same time and some of the eggs are capable of self-fertilization.

Among the better known of the strictly hermaphroditic pelecypods are certain species of *Pecten*, *Gemma*, *Tridacna*, *Tivela*, *Anodonta*, *Kellia*, *Thracia*, and other genera of the suborder Anatinacea, *Cardium*, *Pisidium*, *Sphaerium*, *Musculium*, *Poromya*, *Silenia*, *Cyclas*, and at least one species (*Teredo diegensis*) of the wood-boring bivalves. In a considerable proportion of these the eggs are fertilized within the mantle cavity and the young complete development to the adult form in brood pouches on the gills of the parent.

The ribbed scallop (*Pecten irradians*) may be taken as a typical example of functional hermaphoriditism. In this and other species of the genus the spermatic tissues occupy the dorsal portion of the gonad and the ovarian tissues the much larger ventral portion. The two portions are not demarcated morphologically but are readily distinguished in sexually mature individuals because of the differences in color, the spermatic tissues being creamy white and the ovarian tissues deep pink. Both eggs and spermatozoa are discharged through the same efferent ducts.

Both types of gametes mature at about the same time, although there is a tendency toward protandry, and a considerable portion of the sperm will usually be discharged before any of the eggs are spawned. In other individuals both eggs and sperm are discharged simultaneously and self-fertilization may sometimes occur (Gutsell, 1931). In nature the spawning of one individual, followed by the presence of gametes in the water presumably initiates a corresponding reaction in many other individuals in the vicinity. Simultaneous spawning and cross fertilization will then be general in the population. Furthermore, certain individuals appear to be self-sterile.

In other species of the same genus, as *P. opercularis* and *P. maximus*, some individuals are distinctly protandric at each spawning season, nearly all of the sperm being discharged before the eggs of the same individual become fully ripe. Other individuals are evidently proterogynous. There is thus a brief period when the individual is morphologically hermaphroditic but functionally male and another period when it is functionally female, with an intervening stage when it is functionally hermaphroditic. A similar condition is found in *P. latiauritus* var. *monotimeris* on the coast of Southern California. Protandry is more frequent than proterogyny. This type of sexuality is consequently intermediate between such forms as are strictly hermaphroditic and those that experience consecutive male and female sexual phases.

In one species (*Teredo diegensis*) of the wood-boring mollusks most of the mature individuals are functionally hermaphroditic, following a primary protandric phase. The gonads at all seasons of the year on the coast of Southern California usually contain ripe sperm and mature ova. Experimental self-fertilization is often successful. Larvae are present in the brood chambers of the gills at all seasons of the year. In addition to the larviparous hermaphrodites the population contains a very small percentage of individuals known as true males. In the latter the ovocytes are aborted.

The successive sexual phases of this species may be indicated by the following schematic representation:

Primary male phase . . . True male (exceptional) . . .
Primary male phase . . . Functional hermaphrodite . . .

In a species of cockle (*Cardium corbis*), of the Pacific coast, the eggs and sperm are produced simultaneously in adjacent follicles of the gonads (Edmondson, 1920). This is the case also with *C. crassum* and *C. serratum*, although *C. edule* is dioecious. The Pismo clam (*Tivela stultorum*) is hermaphroditic, but as a general rule the sperm and eggs do not mature at the same time (Weymouth, 1920). Consequently the conditionsare seldom favorable for self-fertilization. There is a general tendency toward protandry, for the hermaphroditic condition of the adult is frequently preceded by a primary male phase in young individuals.

(*b*) *Accidental or developmental.* Even in species which are otherwise strictly of separate sexes there may be an occasional individual with functional hermaphroditism. These can all be considered as resulting from deviations in the developmental processes due to the failure of the sex-differentiating mechanism to function normally. The proportion of spermatogenic and ovogenic tissues in the gonad is highly variable, some individuals having approximately equal parts of both sexual types, while others are principally of one sex, with but a few cells characteristic of the opposite sex. This type of sexuality is more common in the pelecypods than in most other groups of animals and it occurs most frequently in young individuals at the first reproductive season. In certain local races of oviparous oysters, clams, and mussels it is impossible to determine whether the initial sexual phase is normal or accidental hermaphroditism.

2. *Consecutive sexuality*

This type of sexuality implies a single change in the functional sexuality of the individual, or sex reversal. The male and female phases may intergrade or they may be sharply separated. Consecutive sexuality is of wide occurrence, being found not only in most classes of mollusks but in other phyla of invertebrates, including annelids, echinoderms, nemerteans and crustaceans, as well as in some plants and a few fishes. Most of the invertebrates of this type experience but a single change of sex, usually from male to female. An overlapping of the two sexual phases may produce a brief period of hermaphroditism, at which time self-fertilization may occur. Other representatives of the same groups may experience a rhythmical sexuality, with regularly alternating male and female phases.

The histological basis of this type of sexuality depends upon the presence of an essentially ambisexual gonad, associated with a hereditary mechanism which causes the activation of one of the two types of sexual cells in advance of the other.

Consecutive sexuality may become manifest during a single spawning season, one sexual phase following the other without an intervening period of recuperation, or there may be a more or less prolonged period of sexual inactivity between two successive phases. When there is but a single spawning period each year there is often a period of several months between the termination of one sexual phase and the beginning of the next.

The quohog (*Venus mercenaria*) may be taken as an example of consecutive sexuality. In this species, as determined by Loosanoff (1937), nearly all individuals experience a juvenile functional male phase when only a few months of age and only a small fraction of the definitive size. Following this initial male phase the sexes are with few exceptions strictly either male or female, with approximately equal numbers of each sex. There is no indication of a further change of sexuality. The primary, juvenile gonads are invariably more or less distinctly ambisexual, forming a graded series from predominantly male to predominantly female, although about 98 per cent of all individuals are protandric. The remaining 2 per cent are proterogynic. The adults are thus dioecious, following (with few exceptions) a more or less aborted primary male phase.

The sexual conditions in different individuals of this species may be schematized as follows:

1. Juvenile true male phase (exceptional) ... Adult male (true male).
2. Juvenile ambisexual male phase ... Adult male.
3. Juvenile ambisexual male phase ... Adult female (protandric female).
4. Juvenile ambisexual male phase ... Adult hermaphrodite (exceptional).
5. Juvenile female phase (exceptional) ... Adult female.

This type of sexuality is also characteristic of the wood-boring mollusk *Bankia setacea*, although the protandric females may experience a second change of sexual phase if the length of life of the individual is sufficiently prolonged. Self-fertilization is sometimes possible in the juvenile phase of protandric females, due to the overlapping of male and female phases (Coe, 1941). The population contains a small proportion of true males as well as of juvenile females; in the latter the initial male phase is aborted or omitted.

Xylophaga dorsalis, another species of wood-boring pelecypod, is evidently protandric, for most of the smaller and younger individuals function as males and the larger as females (Pynchon, 1941). The change of sex is indicated not only by the hermaphroditic gonad but also by the appearance of the seminal vesicles, which are large in the male phase but diminish in size and eventually disappear in the female phase. This is one of the few pelecypods in which a specialized storage organ for the sperm has been found but there is no direct evidence that self-fertilization is possible.

3. *Rhythmical consecutive sexuality*

Some of the ambisexual species which are composed of individuals living long enough to undergo several complete spawning periods experience an equal number of sexual phases. Such is the case with the larviparous oysters, *Ostrea edulis*, *O. lurida*, *O. equestris* and others. In all of these the initial phase is usually male, followed by a series of alternating female and male phases throughout life. The eggs are fertilized within the mantle cavity, usually with sperm which have been discharged into the water by other individuals in the vicinity. The fertilized eggs are carried through the gills to the pallial chamber of the parent, where they complete development to the adult form.

The European oyster (*Ostrea edulis*) is the best known example of this type of sexuality as the result of the extensive studies by Orton (1927, 1927a, 1933). In this species the initial stages of spermatogenesis are begun even before the ova of the female phase are set free from the gonad. Sperm formation then continues for a month or two. After the sperm have been discharged the production of ova for the following female phase is begun. This phase may become functional immediately or may be delayed until the following year, according to the season. As a general rule under favorable conditions each adult oyster completes one male phase and one female phase each year. Since some individuals function in the male phase early in the spawning season and change later to the female phase, while others have the reverse sequence of the sexual phases, the oyster population is provided with both sexual types during the entire reproductive season (Cole, 1941).

Orton (1927) found much variation in the proportion of the two types of sexual cells in the gonads of different individuals, with all gradations from "pure male" to "pure female." The "pure male" differs from the male-phase individual which has followed the female phase in lacking ovocytes in the gonad and in the profusion of sperm produced.

The Pacific coast oyster (*Ostrea lurida*) experiences a similar series of male and female phases. On the coast of Southern California those individuals which are hatched in early spring may sometimes have three phases of funtional sexuality during one calendar year. A brief male phase when the individual is 4 to 6 months of age is followed by a more lengthy female phase. The latter may be concluded in time for a second male phase in the autumn. Spermatogenesis is usually in progress before the larvae of the female phase have left the mantle cavity.

Individuals which are hatched in the summer or early autumn complete only one or two sexual phases in that calendar year. This individual variation in periodicity provides the population with individuals in both sexual phases at all times during the spawning season (Coe, 1932a, 1934). Two separate spawning periods, resulting in two broods of young, may sometimes occur during a single female phase. Hopkins (1936, 1937) also concluded that in certain years about half the adult oysters in the culture beds in Puget Sound produced two broods of young during the spawning season, while in other years only about three-fourths of the adults produced even a single brood.

In addition to those individuals with regularly alternating sexuality the population contains a small proportion of true males which retain the male phase indefinitely and perhaps throughout life. Furthermore, some of the primary male-phase individuals, the so-called ambisexual males, have a preponderance of ovocytes in the gonad and change to the female phase after a brief initial male phase. Others of the same age have relatively few and small ovocytes in the primary gonad and retain the male phase much longer. And finally, the primary male phase is occasionally aborted or omitted, the first really functional phase thereby becoming female.

The following scheme will illustrate the successive sexual phases of these different classes of individuals:

1. True male (exceptional) . . . Second male phase . . .
2. Ambisexual male phase . . . First female phase . . . Second male phase . . . Second female phase . . . Third male phase, etc.
3. Primary female phase (exceptional) . . . First male phase . . . Second female phase, etc.

Another species, *O. equestris*, with similar sexual phases, is found on the southeastern coast of the United States and on the shores of the Gulf of Mexico. Gutsell (1926) first called attention to its ambisexual nature.

Teredo navalis, one of the species of wood-boring mollusks, likewise has a closely similar sequence of sexual phases. The protandric nature of the sexual cycle and the distinctions between ambisexual males and true males were reported by Coe

(1933). The sexual rhythm from the primary male phase to the female phase, sometimes followed by a second sequence of male and female phases, was described the following year (Coe, 1934a), with an account of the histological changes involved. In a brief summary of the sexual conditions of this species (Coe, 1935) it was shown that in exceptional individuals the initial sexual phase is female. Confirmation of this sequence with additional data was published by Grave and Smith (1936). A statistical analysis of the sexual phases of 2987 individuals examined at various ages and seasons of the year was reported by Coe (1936) as additional proof of the sexual rhythm and the presence in the population of a small proportion of true males and of a still smaller proportion of proterogynic individuals. These conditions were further emphasized in a subsequent paper by Coe (1941). In a study of young individuals only, Grave (1942) concluded that the proportion of proterogynic individuals was greater than previous observations had indicated. The data presented, however, do not seem to justify such a conclusion.

The following scheme will indicate the probable successive sexual changes in such individuals as live long enough to experience what may be considered as the normal sequence of sexual phases. Mortality during the second phase is usually very high and few individuals reach the fourth (female) phase.

1. True male phase (exceptional) ... Second male phase ...
2. Ambisexual male phase ... First female phase ... Second male phase ... Second female phase (if length of life suffices).
3. Functional hermaphroditic phase (exceptional) ... Male or female phase ...
4. Female phase (exceptional) ... First male phase ... Second female phase ...
5. Female phase (exceptional) ... Second female phase (?)

4. *Alternative sexuality*

In the oviparous oysters and perhaps in some other pelecypods the adults function seasonally as separate sexes, although it is impossible to predict during one reproductive season the sexual phase which the individual will assume at the next.

This type of alternative sexuality is illustrated by the commercial oyster of the North Atlantic coast, *Ostrea virginica*. At the first spawning season there is a strong tendency toward protandry, more than 70 per cent of the young oysters in some localities functioning as males at that time (Coe, 1938). After the second spawning season the number of individuals in each sexual phase is approximately equal, with a tendency toward an excess of females among the older individuals (Needler, 1936).

The adult Japanese oyster (*O. gigas*) was proved by Amemiya (1929) to be subject to a change in sexuality in the interval between two spawning seasons. A corresponding condition was found by Needler (1932), Burkenroad (1937), and Galtsoff (1937, 1938) in *O. virginica*.

Galtsoff (1937, 1938), using marked adult oysters, found that 8 per cent of those functioning as males changed to the female phase the following year, while 13.1 per cent of the females similarly reversed their sex. In the second year 11.2 per cent of the males of the preceding year showed a change of sex and 12.1 per cent of the females changed to the male phase. The total number of sex reversals in the first year was 9.7 per cent and in the second year 11.5 per cent. Hence this process of reversing the sexual phase seemed to be limited to a minority of the experimental animals. Moreover, 69 per cent of those that changed the sexual phase in the first year reversed again the second year. Some of the males which had changed to the female phase spawned in male fashion in the early part of the season but as typical females later.

In *O. gigas*, however, the proportion of the adult population experiencing changes in sexuality is evidently much greater, for Amemiya (1929) concluded that 25 per cent of the females and 60 per cent of the males in his experiment reversed their sex during one winter.

Protandry and change of sexual phase has been found in other species of oviparous oysters. Roughley (1933) concluded that nearly all the young of *O. cucullata* (*commercialis*) spawn first as males, although there appeared to be 2.7 times as many females as males in the adult population. Needler (1936) found more than 3 times as many females as males in an old population of *O. virginica*.

The primary gonad of *O. virginica* usually contains the antecedent cells of both sexes. Its protandric nature frequently becomes manifest when very young by the rapid proliferation of the spermatogonia and the formation of few or many ovocytes (Coe, 1932, 1934, 1938). In Long Island

Sound the process of gametogenesis often begins in the autumn when the animal is only 3 to 4 months of age; it is interrupted at the approach of winter and resumed the following spring. The first spawning occurs at the age of about one year.

The gonads of the young oysters previous to sexual maturity show all gradations between those of the so-called true males in which no ovocytes can be detected and those which develop directly into ovaries. Approximately 1 per cent (61 of 6315) of these young oysters become functionally hermaphroditic at the first spawning season. Most of the others function strictly as males or females during the first spawning season, although an occasional individual may produce sperm during the early part of the season and ova a few weeks later (Coe, 1938).

In those young individuals that are to function as males during their first spawning season, such ovocytes as may be formed in the early primary gonad are cytolyzed during the progress of spermatogenesis. Female sexual differentiation may be direct, with little or no indication of protandry, but it is more often indirect. In the latter case the primary gonad is more or less distinctly ambisexual during the autumn but takes on the appearance of an ovary the following spring by the proliferation of ovogonia and the cytolysis of the spermatogenic cells.

"A subsequent change from female to male phase evidently results from the later activation of some of the descendants of the primary undifferentiated gonia into spermatognia. The propagation of such undifferentiated gonia may be continued year after year or even through a long lifetime, since some residual gonia always remain after spawning" (Coe, 1938). The process by which this occurs is more fully explained by Loosanoff (1942).

The following scheme shows the presumable sexual phases of this species of oyster, based on the assumption that only those in which the primary gonad is ambisexual experience a change of sex in the second and later years.

1. Primary gonad male ... Male phase (first year) ... True male ...
2. Primary gonad ambisexual ... Male phase (first year) ... Male or female phase ...
3. Primary gonad ambisexual ... Functional hermaphrodite (first year) exceptional ...
4. Primary gonad ambisexual ... Male phase (early portion of first year); female phase (later portion of first year) exceptional ...
5. Primary gonad ambisexual ... Male phase (first year) ... Female phase (second year) ... Male or female phase ...
6. Primary gonad ambisexual ... Female phase (first year) ... Female (or male?) phase ...
7. Primary gonad female ... Female phase (first year) ... True female ...

Immediately after each spawning period the residual differentiated sexual cells undergo phagocytosis and cytolysis, leaving only undifferentiated gonia in the collapsed gonadal follicles. The individual thereby returns to a condition similar to that of sexual immaturity. Within a few weeks however, the apparently undifferentiated gonia resume activity and they then show that they have, in reality, become uniformly differentiated into the cells characteristic of the one sexual phase or the other. It is not at present known to what extent, if any, environmental conditions determine whether this differentiation shall be in the male or female direction.

The percentage of females at the first spawning season varies with the locality and with the season. The females at this time average somewhat larger than males of the same age and the data obtained as to the sexuality of more than 6000 young oysters from various localities from Cape Cod to the Gulf of Mexico indicates that those conditions and situations most favorable for rapid growth have the largest proportion of females. Examination of the gonads of 4033 yearling oysters from Long Island Sound whose sex could be determined indicated that 3578 were in the juvenile male phase and 416 in the female phase, together with 39 which were evidently hermaphroditic. This is a ratio of 11.6 females to 100 males. Different samples varied from 3.34 to 25 (Coe, 1938).

There were in the same collections, however, 406 individuals which were at the time sexually undifferentiated. Since female sexual differentiation commonly occurs at a later period than male differentiation, it seems probable that a much larger proportion of the sexually undifferentiated young would have developed into females than was found among those which were already sexually differentiated at the time of examination. The proportion of females is always largest toward the end of the spawning season, due in part to the protandric nature of seasonal hermaphrodites but especially to the delayed differentiation of females.

If allowance be made for these conditions it seems safe to assume from the data given by Coe (1938) that the yearling population of oysters in

Long Island Sound consists in average years of approximately 20 individuals in the functional female phase to 100 in the male phase.

A sample of 751 yearlings from Delaware Bay had a ratio of 41.92 females to 100 males, while three samples from Beaufort, North Carolina, varied only from 37.09 to 48.84 females to 100 males.

Whether these differences may be due to differences in the hereditary characteristics of the populations in the localities mentioned or whether to responses to different nutritive and other environmental conditions, or to both, is not at present known.

Since there is usually found an equality in the ratio of the sexes or an excess of females after the second year, it is obvious that at least 25 per cent of the juvenile males in these localities must later function seasonally as females. It is not known whether any of the juvenile females later change to males. Nor is it known to what extent environmental conditions influence the sex differentiating mechanism.

B. UNISEXUALITY; DIOECISM; GONOCHORISM

The vast majority of pelecypods are believed to be strictly of separate sexes, except for an occasional hermaphrodite. Such was formerly the conclusion in regard to *Venus mercenaria* and *Ostrea virginica* until their juvenile sexual phases, as has already been explained, were discovered in recent years. Consequently it is now evident that both juveniles and adults must be examined in order to ascertain definitely the sexual conditions of a species.

This has now been done for *Volsella* (*Modiolus*) *demissus*, *Mya arenaria*, *Petricola pholadiformis*, *Barnea truncata*, *Mytilus californianus*, *M. edulis*, *Septifer bifurcatus*, *Donax gouldii* and *Anomia simplex* without findings evidence of ambisexuality except in the sexually undifferentiated young gonads and an occasional hermaphrodite. None of these species has a distinctly juvenile phase of sexuality in the localities in which the investigations were made. Males and females occur in approximately equal numbers.

DISCUSSION

The inquiry may now be raised as to the significance of the diversities of sexuality that have been mentioned. Is the sex-differentiating mechanism so labile that it responds to relatively slight environmental changes or are variable genetic combinations more directly responsible for the variability observed? The answer seems to be that both these factors are presumably operative in all cases, although the evidence as to the influence of the environment requires more direct experimental proof.

In general, it is evident that there is often an inherent tendency toward a change in functional sexuality at successive reproductive periods. This change may occur during the brief interval between two successive spawnings in one season, as in the larviparous oysters, in *Teredo* and in *Pecten*, or in successive seasons, as in the oviparous oysters. There may be a single change, affecting the primary male phase only, as in the consecutive sexuality of *Venus* and as is usual in *Bankia* or the changes may be numerous, as in the long-lived larviparous oysters.

Juvenile sexuality, or neoteny, as reported for *Venus* and oviparous oysters is of wide occurrence in both animals and plants, being characteristic of certain gastropods (as *Crepidula*), annelids (as *Ophryotrocha*), gephyreans (as *Bonellia*), echinoderms (as *Leptasterias*), nemerteans (as *Geonemertes*), and has been reported for crustaceans (*Pandalus*), fishes (*Sparus*), and amphibians (*Rana*). It is, of course, well known for the axolotyl. Some of these resemble the pelecypods mentioned in that the sexuality of certain individuals in the juvenile phase is opposite that of the adult. Both in the pelecypods and in the other groups mentioned the neotenous condition is often abortive, leading to the cytolysis of such gametes as may be partially or fully formed; hence spawning may not occur in the juvenile phase.

Particularly noteworthy is the variability in the sexuality of different individuals of the same species and in some cases in a single individual at different periods of life. This variability resembles in some respects the color patterns of certain mammals or of certain plants. Both are evidently the visible manifestations of various combinations of multiple modifying hereditary factors.

In the functionally hermaphroditic species, as *Pecten*, for example, the relative extent of the spermatic and ovarian tissues in the gonad is highly variable. Some individuals closely approach a unisexual condition, particularly a female condition, with only a very small amount of spermatic tissue or none at all. But occasionally the relations are reversed. Furthermore the extent of protandry or of proterogyny varies greatly in different members of a single population.

The importance of environmental influences in modifying these characteristics has not been satisfactorily shown experimentally for the pelecypods, although Amemiya (1935) found that groups of Japanese oysters having portions of their gills removed showed a larger percentage of males the following season than similar groups with uninjured gills. Awati and Rai (1931) also concluded that the disturbance in the nutrition of the Bombay oyster caused by the commensal crab *Pinnotheres* increased the proportion of males and Rosenwald (1926) found that the gastropod *Limax* became protandric or proterogynic according to the environmental conditions, particularly the amount of moisture. It was mentioned that the Virginia oyster has a larger proportion of females in the yearling population in those years and in those localities most favorable for rapid growth (Coe, 1936, 1938). At this age the functional females are of larger average size than those individuals that are functioning as males. Hence it seems quite possible that temperature and nutritive conditions within the body may influence the proliferation of one rather than the other of the alternative types of sexual cells in the early ambisexual gonad. Such influences are well authenticated among nematodes, annelids, amphibians and plants.

In several groups of animals, both invertebrates and vertebrates, as well as in some plants, there exist local races with differing hereditary types of sexuality. Such races are reported by Bloomer (1939) in the fresh-water pelecypod *Anodonta cygnea*, and have been thought to exist in the Virginia oyster (Coe, 1936, 1938). Additional information, particularly of an experimental nature, will presumably reveal them in many other species.

CONCLUSION

It may be concluded then that the pelecypods as a group are predominantly of separate sexes. Among the relatively few hermaphroditic species four intergrading types of ambisexuality are recognized: functional ambisexuality (functional hermaphroditism), consecutive sexuality, rhythmical consecutive sexuality and alternative sexuality. Some of the hermaphroditic species are characterized by multiple modifying hereditary sex factors which may produce all grades of ambisexuality from true males to true females.

There are also various hereditary physiological time factors which control the periodicities of the alternative sexual phases. The ambisexual species show a general tendency toward a rhythmical series of alternating male and female phases, of which the initial sexual phase is more frequently male. Some of the species appear to be composed of local races with differing sexual tendencies and these tendencies become manifest in differing degrees according to the environmental conditions.

The sexual conditions described on the foregoing pages are not peculiar to the pelecypods but have their parallels in other groups of organisms, both animals and plants. Even in the sponges there are species with incompletely separated sexes, some individuals functioning for a time as males and others as females, while the later formation of eggs in predominantly male individuals suggests a general tendency toward protandry (Fincher, 1940). Such is also the case with certain planarians, gephyreans, nematodes, annelids, nudibranchs, gastropods, echinoderms, crustaceans, and fishes. In some of these, as has been mentioned for *Venus*, the fully mature individuals are strictly of separate sexes, although nearly all the young first function as males. The genetic males (true males) continue to function as such throughout life, while the genetic females (hermaphroditic males, or protandric females) function as males when young and as females thereafter.

There is no direct proof of phenotypic sex determination in the pelecypods, although as a general rule in cases of alternative sexuality the male phase is often indicative of immaturity and unfavorable environmental influences, while the female phase is more often associated with optimum physiological conditions. This is in harmony with the evidence from other groups of animals and plants.

All these manifestations of sexuality are consistent with the view that the prospective sexuality of the individual is dependent upon the quantitative balance of the male as opposed to the female factors in the hereditary mechanism. They may all be referred to the homozygosity or heterozygosity of the primary sex factors, with their associated modifying factors for the activation or suppression of either the male- or female-determining components at certain stages of development or in harmony with different degrees of physiological maturity. They are also responsive to environmental conditions both within and without the body of the organism.

LIST OF LITERATURE

AMEMIYA, I. 1929. On the sex change of the Japanese common oyster, *Ostrea gigas* Thunberg. *Proc. Imp. Acad. Tokyo*, 5: 284–286.

——. 1935. Effect of gill excision upon the sexual differentiation of the oyster (*Ostrea gigas*). *Rept. Jap. Ass. Adv. Sci.*, 10: 1023–1026.

AWATI, P. R., and H. S. RAI. 1931. *Ostrea cucullata* (the Bombay oyster). *Indian Zool. Memoir*, No. 3, Lucknow.

BLOOMER, H. H. 1939. A note on the sex of *Pseudanodonta* and *Anodonta*. *Proc. Malac. Soc. London*, 23: 285–297.

BURKENROAD, M. D. 1931. Sex in the Louisiana oyster, *Ostrea virginica*. *Science*, 74: 71–72.

——. 1937. The sex-ratio in alternational hermaphrodites, with especial reference to the determination of sexual phase in oviparous oysters. *Jour. Marine Research*, 1: 75–84.

COE, W. R. 1932. Sexual phases in the American oyster (*Ostrea virginica*). *Biol. Bull.* 63: 419–441.

——. 1932. Development of the gonads and the sequence of the sexual phases in the California oyster (*Ostrea lurida*). *Bull. Scripps Inst. Oceanogr., Tech. Ser.*, 3: 119–144.

——. 1933. Sexual phases in *Teredo*. *Biol. Bull.*, 65: 283–303.

——. 1934. Alternation of sexuality in oysters. *Am. Nat.*, 68: 236–252.

——. 1934a. Sexual phases in the pelecypod mollusk *Teredo*. *Science*, 80: 192–193.

——. 1935. Sequence of sexual phases in *Teredo*, *Ostrea* and *Crepidula*. *Anat. Rec.*, Suppl., 64: 81.

——. 1936. Sequence of functional sexual phases in *Teredo*. *Biol. Bull.*, 71: 122–132.

——. 1936a. Sex ratios and sex changes in mollusks. *Mem. Mus. Hist. Nat. Belgique* (2 ser.), fasc. 3: 69–76.

——. 1938. Primary sexual phases in the oviparous oyster (*Ostrea virginica*). *Biol. Bull.*, 74: 64–75.

——. 1940. Divergent pathways in sexual development. *Science*, 91: 175–182.

——. 1941. Sexual phases in wood-boring mollusks. *Biol. Bull.*, 81: 168–176.

——. 1943. Development of the primary gonad and differentiation of sexuality in *Teredo navalis* and other pelecypod mollusks. *Biol. Bull.*, 84: (In press).

COE, W. R., and HARRY J. TURNER, Jr. 1938. Development of the gonads and gametes in the soft-shell clam (*Mya arenaria*). *Jour. Morph.*, 62: 91–111.

COLE, H. A. 1941. The fecundity of *Ostrea edulis*. *Jour. Mar. Biol. Ass'n.*, 25: 243–260.

EDMONDSON, CHARLES H. 1920. Edible mollusca of the Oregon coast. *Occ. Pap. Bishop Mus.*, 7: No. 9.

FINCHER, JOHN ALBERT. 1940. The origin of the germ cells in *Stylotella heliophila* Wilson (Tetraxonida). *Jour. Morph.*, 67: 175–197.

GALTSOFF, PAUL S. 1937. Observations and experiments on sex change in the adult American oyster, "*Ostrea virginica*." *Collecting Net*, 12: 187.

——. 1938. Sex change and physiological intersexuality in *Ostrea virginica*. (Abstr.) *Anat. Rec.*, Suppl., 72: 42.

GRAVE, BENJAMIN H. 1942. The sexual cycle of the shipworm, *Teredo navalis*. *Biol. Bull.*, 82: 438–445.

GRAVE, BENJAMIN H., and JAY SMITH. 1936. Sex inversion in *Teredo navalis* and its relation to sex ratios. *Biol. Bull.*, 70: 332–343.

GUTSELL, J. S. 1926. A hermaphroditic viviparous oyster of the Atlantic Coast of North America. *Science*, 64: 450.

——. 1931. Natural history of the bay scallop. *Bull. Bureau Fish.*, U. S. Dept. Commerce, 46 (1930): 569–632.

HOPKINS, A. D. 1936. Ecological observations on spawning and early larval development in the Olympic oyster (*Ostrea lurida*). *Ecology*, 17: 551–566.

——. 1937. Experimental observations on spawning, larval development and setting in the Olympic oyster (*Ostrea lurida*). *Bull. U. S. Bureau Fish.*, 23: 439–503.

LOOSANOFF, VICTOR L. 1937. Development of the primary gonad and sexual phases in *Venus mercenaria* Linnaeus. *Biol. Bull.*, 72: 389–405.

——. 1942. Seasonal gonadal changes in the adult oysters, *Ostrea virginica*, of Long Island Sound. *Biol. Bull.*, 82: 195–206.

NEEDLER, ALFREDA B. 1932. Sex reversal in *Ostrea virginica*. *Contr. Can. Biol. and Fish.*, 7: 285.

——. 1936. Sex ratios in oysters of known age. *Prog. Rep. Atlantic Biol. Sta.*, 7: No. 49.

ORTON, J. H. 1927. Observations and experiments on sex-change in the European oyster (*O. edulis*). *Jour. Mar. Biol. Ass'n.*, 14: 967–1045.

——. 1927a. A note on the physiology of sex and sex determination. *Jour. Mar. Biol. Ass'n.*, 4: 1047–1055.

——. 1933. Observations and experiments on sex change in the European oyster (*O. edulis*). Part III, On the fate of unspawned ova. Part IV, On the change from male to female. *Jour. Mar. Biol. Ass'n.*, 6: 1–54.

PELSENEER, PAUL. 1935. Essai d'ethologie d'après l'étude des Mollusques. *Pub. Fond. Agathon De Potter; Acad. Roy. Belgique, Cl. Sci.*, Bruxelles, 1–662.

Pynchon, R. Denison. 1941. On the biology and relationships of the lamellibranch *Xylophaga dorsalis* (Turton). *Jour. Mar. Biol. Ass'n.*, 25: 1–39.

Rosenwald, K. 1926. Beeinflussung des Geschlechts von *Limax laevis*. *Zeits. f. ind. Abs. u. Verer.*, 43: 238–251.

Roughley, T. C. 1933. The life history of the Australian oyster (*Ostrea commercialis*). *Proc. Linnean Soc. New South Wales*, 58: 279–333.

Weymouth, Frank W. 1923. The life history and growth of the *Pismo* clam. *California Fish and Game*, Bull. 7: 1–120.

Contribution No. 198

THE RATIO OF CARBON DIOXIDE CONSUMPTION TO OXYGEN EVOLUTION IN SEA WATER IN THE LIGHT[1]

By

MARSTON C. SARGENT AND JAMES C. HINDMAN

Scripps Institution of Oceanography
University of California
La Jolla, California

The production of organic matter in sea water by the phytoplankton community has frequently been computed (Nielsen, 1937; Riley 1941) by measuring the oxygen evolution in bottled sea water samples and calculating the organic matter on the assumption that the phytoplankton organisms produced glucose by photosynthesis according to the equation:

$$6CO_2 + 6H_2O \rightleftharpoons C_6H_{12}O_6 + 6O_2.$$

According to this assumption CO_2/O_2, that is, the ratio of carbon dioxide consumed to oxygen evolved in the light, is 1.0. Thompson and Gilson (1937, p. 31) have pointed out that as phytoplankton tissue contains nitrogen and carbon atoms in the ratio of one to seven, one fifth of the oxygen produced must originate from nitrate and the ratio CO_2/O_2 should be 0.8. On the other hand, Redfield (1934, p. 182) found that in the oxidation of organic matter in deep water the ratio of the carbon dioxide produced to the oxygen consumed was 1.17, but he considered the accuracy of the determination low.

In the experiments described below, the consumption of carbon dioxide and the formation of oxygen were both measured on the same samples of sea water exposed to natural illumination for a day.

Sea water was collected directly in a twelve liter Pyrex bottle, and two 4600 ml bottles with tight stoppers were completely filled from this reservoir through a siphon. Usually two collections were made and two pairs of bottles filled at a time. One bottle of each pair was wrapped in several layers of khaki duck and the four bottles were hung from the end of the pier in a frame at a depth below the surface varying from one to three meters according to the stage of the tide, and almost five meters from the bottom. After twenty-four hours the bottles were removed to a dark constant temperature room kept usually at 23° ± 0.5° C. (somewhat warmer than the sea), and allowed eighteen hours to come to temperature equilibrium.

---

[1] Contributions from the Scripps Institution of Oceanography, New Series, No. 198.

Reprint from SEARS FOUND. JOURN. MAR. RES.

The concentration of carbon dioxide was calculated from a measurement of the pH using the data of Moberg, Greenberg, Revelle, and Allen (1934, p. 269). The pH measurements were made in a vessel of about 250 ml capacity arranged so that it could be filled from the experimental bottle through a siphon and emptied through another siphon. In this way the vessel could be repeatedly rinsed and filled with a minimum contact of the water with air. The pH was measured electrometrically by means of a glass electrode with a silver-silver chloride reference electrode. The latter was prepared by electrolytically plating a silver wire with silver chloride in a sodium chloride solution.

The silver-silver chloride electrode was used in preference to a calomel electrode because of the uncertain junction potentials often characteristic of the latter. More comprehensive data on the use of these electrodes in sea water is to be published at a later date. The measuring circuit consisted of a Leeds and Northrup type K potentiometer in conjunction with a Beckman No. 1100 electrometer. The precision of measurement when corrections were made for small temperature differences between the pairs of samples was approximately ± 0.1 mv or ± 0.0017 pH for each pair. In two cases the differences between light and dark bottles were so great that measurements were made with a Beckman pH meter.

After the completion of the electrometric measurement, samples were siphoned into 150 ml bottles and the oxygen concentration was measured by the Winkler method. The standard deviations of differences between the means of triplicate titrations varied from 0.02 to 0.07 milliliters of oxygen per liter.

In the table are shown the data for nine sets of observations. The mean of the nine resulting figures for the CO_2/O_2 ratio is 0.95 which, in view of the relatively large errors involved in the measurement of these small quantities, cannot be considered as significantly different from 1.0. With these data on the activities of raw sea water in the light can be compared Barker's (1935) data showing that the photosynthetic quotient of a pure culture of *Nitzschia closterium* lay between 0.93 and 0.96. The value found by Barker for this diatom appears from our measurements to be characteristic of the whole phytoplankton community. Both Barker's data and ours lead to the conclusion that fats constitute only a small part of the products of the photosynthesis of marine phytoplankton. In the practical problem of calculating the production of organic matter in the sea from observations on oxygen evolution the uncertainties are so much greater than this 5% difference that the ratio 1.0 can be used without hesitation.

MEASUREMENTS OF CARBON DIOXIDE CONSUMED AND OXYGEN EVOLVED BY RAW SEA WATER

	Temperature in °C	Potential in millivolts	Difference in millivolts	Difference in alkalinity		Difference in CO_2 concentration ml./l.	Concentration of O_2 ml./l.	Difference ml./l.	Ratio of CO_2 consumed to O_2 evolved
				pH	ΔpH				
July 20 light		−35.11					5.44		
" " dark	22.9	−33.61	1.50		0.0255	0.34	5.11	0.33	1.03
" " light		−35.00					5.40		
" " dark	22.8	−33.98	1.02		0.0174	0.23	5.11	0.29	0.79
" 25 light		−36.85					5.00		
" " dark	22.8	−35.60	1.25		0.0213	0.28	4.68	0.32	0.88
" " light		−36.11					5.03		
" " dark	22.8	−35.23	0.88		0.015	0.20	4.81	0.22	0.91
" 28 light		−35.85					5.30		
" " dark	22.8	−35.50	0.35		0.0060	0.08	5.17	0.13	0.6
Aug. 20 light		−30.94					5.02		
" " dark	19.5	−30.40	0.54		0.0093	0.12	4.88	0.14	0.88
" " light		−31.22					5.03		
" " dark	19.5	−30.75	0.59		0.0101	0.13	4.91	0.12	1.1
Sept. 29 light				8.78			8.04		
" " dark	20.8			8.56	0.22	4.6	4.27	3.77	1.2
" " light				8.73			9.00		
" " dark	20.8			8.58	0.15	3.2	6.25	2.75	1.2

The correction suggested by Thompson and Gilson is probably not practically significant with the techniques of measuring organic production in their present condition. However, it is worth considering in the present connection.

In Barker's experiments using artificially dense populations of diatoms the amount of nitrate present initially was so small that the total nitrate reduction was negligible compared with the total carbon dioxide reduction during the period of the observations. Our observations on raw sea water were made late in the season when regeneration of nitrate was presumably occurring nearly or quite as fast as nitrate reduction. If this is true, careful observations should show an annual cycle in the value of CO_2/O_2.

The two nearly identical values found on September 29 merit special consideration. They were obtained during a period of red water caused by a bloom of a *Gonyaulax* sp. when the carbon dioxide consumption and oxygen evolution were about ten times as large as during the other observations. The uncertainty of the values for CO_2/O_2 is not more than $\pm$ 5%. Another feature of the conditions at this time is that the total photosynthesis (difference in final oxygen content between the light bottle and the dark bottle) was only about 10% higher than the respiration (difference between the initial and final oxygen contents of the dark bottle) while during the other observations it was two to four times the respiration. Under these conditions such high values for the ratio CO_2/O_2 could occur if (1) the product of photosynthesis were a carbohydrate, and, (2) the product of respiration were in part a keto-acid, hydroxy-acid or other highly oxygenated substance. If the uncertainty of the values of the ratio CO_2/O_2 in these sets of observations were great, the hypothesis would not be worth formulating, but under the circumstances there is a strong presumption that a fermentation of the kind described was actually occurring on a measurable scale.

CONCLUSIONS

1. The ratio of carbon dioxide consumed to oxygen evolved in the photosynthetic processes of the phytoplankton of the sea is not significantly different from 1.0 during the season of high phytoplankton productivity.

2. Fermentative processes resulting in incomplete oxidation products of carbohydrates may occur on a measurable scale when the organic content of sea water is high.

REFERENCES

BARKER, H. A.
1935. Photosynthesis in Diatoms. Arch. f. Mikrobiol. *6:* 141–156.
MOBERG, E. G., GREENBERG, D. M., REVELLE, R., AND ALLEN, E. C.
1934. The Buffer Mechanism of Sea Water. Bull. Scripps Inst., Ocean. Univ. Calif. Tech. Ser., *3:* 231–278.
NIELSEN, E. S.
1937. The Annual Amount of Organic Matter Produced by the Phytoplankton in the Sound off Helsingfor. Medd, Komm. f. Danmarks Fisk.-og Havundersøgelser. Ser. Plankton, *3*, No. 3: 1–37.
REDFIELD, A. C.
1934. On the Proportions of Organic Derivatives in Sea Water and Their Relation to the Composition of Plankton. James Johnston Memorial Volume, Liverpool, pp. 176–192.
RILEY, G. A.
1941. Plankton Studies. III. Long Island Sound. Bull. Bing. Ocean. Coll. *7:* 1–93.
THOMPSON, E. F. AND GILSON, H. C.
1937. Chemical and Physical Investigations. Sci. Rep., John Murray Exped. *2:* 15–81.

Contribution No. 199

Made in the United States of America

Reprinted from JOURNAL OF BACTERIOLOGY
Vol. 45, No. 6, June, 1943

BACTERIAL UTILIZATION OF LOW CONCENTRATIONS OF ORGANIC MATTER[1]

CLAUDE E. ZOBELL AND CARROLL W. GRANT[2]

Scripps Institution of Oceanography, University of California, La Jolla

Received for publication January 11, 1943

Although several investigators have studied the effect of concentration of organic nutrients upon the growth of microörganisms, most of the experiments have been concerned with concentrations which are much higher than those which occur in soil solutions, sea water and other natural environments. According to Rahn (1932) the concentration of food does not influence the rate of growth of bacteria except when the concentration is very low, 0.01 to 0.1 per cent. Penfold and Norris (1912), Hucker and Carpenter (1927), Friedlein (1928), Bigger (1937) and others report that the minimum concentration of organic nutrients required for the multiplication of heterotrophs ranges from 0.001 to 0.01 per cent or 10 to 100 mgm./l.

That many bacteria multiply and are otherwise physiologically active in very dilute nutrient solutions is manifest from the abundance of bacteria in lake water (Henrici, 1939) and sea water (Benecke, 1933). The organic content of sea water is generally less than 5 mgm./l., yet during the storage of sea water in the laboratory the bacterial population usually increases from a few hundred to several million bacteria per ml. (Waksman and Carey, 1935a). Bacterial multiplication is accompanied by the evolution of carbon dioxide, oxygen consumption, ammonia production, nitrate reduction and other biochemical changes (ZoBell and Anderson, 1936). Similar changes have been noted in lake water (ZoBell, 1940) which contained less than 10 mgm. of organic matter per liter. Butterfield (1929) found that after an extended lag period *Aerobacter aerogenes* multiplied slowly in a solution containing only 0.5 mgm./l. each of glucose and peptone. Heukelekian and Heller (1940) found that when glass beads were present to augment the area of solid surface, *Escherichia coli* grew in either glucose or peptone solutions as dilute as 0.5 mgm./l.

Recently ZoBell and Grant (1942) reported that marine bacteria multiply in mineral media when the concentration of organic nutrients is less than 0.1 mgm./l. There were indications that lower concentrations might provide for bacterial activity. The following experiments were designed to determine the effect of the concentration of various kinds of organic matter upon its rate of utilization by bacteria.

---

[1] Contribution from the Scripps Institution of Oceanography, New Series No. 199.

[2] Now at Brooklyn College, Brooklyn, N. Y. Assisted by Grant No. 555 from the American Philosophical Society.

EXPERIMENTAL METHODS

Since it is a physiologically balanced salt solution which provides for the mineral requirements of all microörganisms which have been tested, aged sea water was used in the preparation of the nutrient solutions. It must be diluted 1:10 to 1:20 when used for fresh-water or soil bacteria but it is satisfactory without dilution for marine bacteria (ZoBell, 1941). The organic content of sea water was reduced to a fraction of a milligram per liter by "ageing" it in the dark for several months at room temperature. The water was collected from unpolluted areas in the sea in glass receptables and passed through a fine fritted glass filter to remove particulate material. After five or six months storage the utilizable organic matter content was found to be around 0.2 mgm./l., and by successively transferring the water to acid-cleaned bottles, autoclaving, reinoculating with a few bacteria and incubating the water, the organic content could be reduced to 0.02 mgm./l.

Bacto-peptone to give concentrations ranging from 0.1 to 100 mgm./l. (0.000,01 to 0.01 per cent or 0.1 to 100 p.p.m.) was added to the water. The media were dispensed in 10 ml. quantities in test tubes, sterilized in the autoclave and inoculated with a 2 mm. loopful containing 10 to 100 bacteria suspended in organic-matter-free water. The inoculated media were incubated at 22°C. and examined daily for evidence of multiplication. Plating procedures revealed that all thirty of the different species of common marine bacteria under investigation multiplied in all concentrations of peptone used. Little or no multiplication occurred in the control medium to which no peptone was added. There was not the faintest clouding of the media containing less than 10 mgm./l. of peptone although most of the cultures clouded the medium which contained 100 mgm./l. of peptone. Eight of the cultures produced a perceptible turbidity in the medium containing 10 mgm./l. of peptone.

Similar results were obtained with glucose. When glucose and other non-nitrogenous substrates were used, 1 mgm./l. of ammonium phosphate was added to the water to provide a source of nitrogen.

It becomes obvious from a few simple calculations that one could not expect media containing less than 10 mgm./l. of organic matter to be clouded by the growth of bacteria. If quantitatively converted into bacterial protoplasm, 1.0 mgm. of organic carbon would yield around 10^{10} bacteria. A medium containing 1.0 mgm./l. of organic carbon could yield no more than 10^7 bacteria per ml. Actually the number would be less because, as will be discussed below, only part of the organic matter is converted into bacterial protoplasm. Since it requires 10^7 to 10^8 bacteria to render a clear medium perceptibly turbid, it follows that ordinary turbidimetric methods are not applicable to the study of bacterial multiplication in dilute nutrient solutions. This may explain why certain workers have reported that bacteria do not multiply in dilute nutrient solutions.

OXYGEN CONSUMPTION

The effect of concentration of organic matter upon the rate of its utilization by bacteria was investigated by noting oxygen consumption. Concentrations of glucose ranging from 0.25 to 100 mgm./l. were added to organic-matter-free water inoculated with a few hundred bacteria per ml. After thorough shaking to provide for uniformity in composition and to insure its saturation with oxygen, the water was dispensed in 60 ml. glass-stoppered bottles. The oxygen content of the water in duplicate pairs of bottles was determined at once, using a modified Winkler technique which was accurate to ±0.01 mgm./l. The oxygen content of the remaining bottles of inoculated water was determined after different periods of incubation in the dark, in a water bath at 22°C. From these data the amount of oxygen consumed by bacteria after different periods of time in the presence of different concentrations of glucose was calculated after making corrections for the controls. The results of a representative experiment are summarized in table 1.

TABLE 1

Amount of oxygen consumed by marine bacteria in water containing different concentrations of glucose after different periods of incubation at 22°C.

PERIOD OF INCUBATION	INITIAL CONCENTRATION OF GLUCOSE IN MGM./L.							
	0.25	0.50	1.0	2.5	5.0	10.0	100	1000
hours	*mgm./l.*	*mgm./l.*	*mgm./l.*	*mgm./l.*	*mgm./l.*	*mgm./l*	*mgm./l.*	*mgm./l.*
21	0.01	0.02	0.02	0.02		0.13	0.03	0.24
45	0.17	0.27	0.20	0.59	0.99	1.78	2.35	7.13
57					1.97	3.51	7.79	8.85*
69	0.19	0.31	0.39	1.05	2.21	4.20	8.85*	
95	0.19	0.33	0.52	1.50	2.42	4.72		
168	0.18	0.34	0.63	1.49	2.80	5.08		

* All of the dissolved oxygen was utilized.

Similar results were obtained with peptone as shown graphically by figure 1. In either peptone or glucose solutions the rate of bacterial activity, as indicated by oxygen consumption, is more or less directly proportional to the initial concentration of substrate. Plate counts indicate that the rate of bacterial multiplication as well as the rate of oxygen consumption is a function of the concentration of the substrate. However, we do not have sufficient data to appraise quantitatively the effect of concentration of substrate upon multiplication and respiration. It has not been feasible to prepare suspensions of "resting cells" which are sufficiently free of oxidizable organic matter to test the effect of concentrations of organic substrates, which range from 0.25 to 10 mgm./l., upon bacterial respiration. Geiger-Huber (1934) found that the rate of respiration of yeasts was proportional to the concentration of glucose until the latter exceeded M/500, above which the rate was independent of the glucose concentration. However, the lowest concentration of glucose tried by Geiger-Huber was M/10,000 or 18 mgm./l.

It is significant that while bacteria multiply more rapidly in the higher concentrations, the substrate disappears first in media containing the lowest initial concentration of the substrate. This is illustrated by the data in table 2 in which the per cent of the glucose oxidized is given, calculated upon a basis of 1.07 mgm. of oxygen being required to oxidize 1.0 mgm. of glucose to carbon dioxide and water. It will be observed that more than half of the glucose was oxidized in five days or less when the initial concentration was less than 1 mgm./l., whereas progressively longer periods of time were required to oxidize half of higher concentrations.

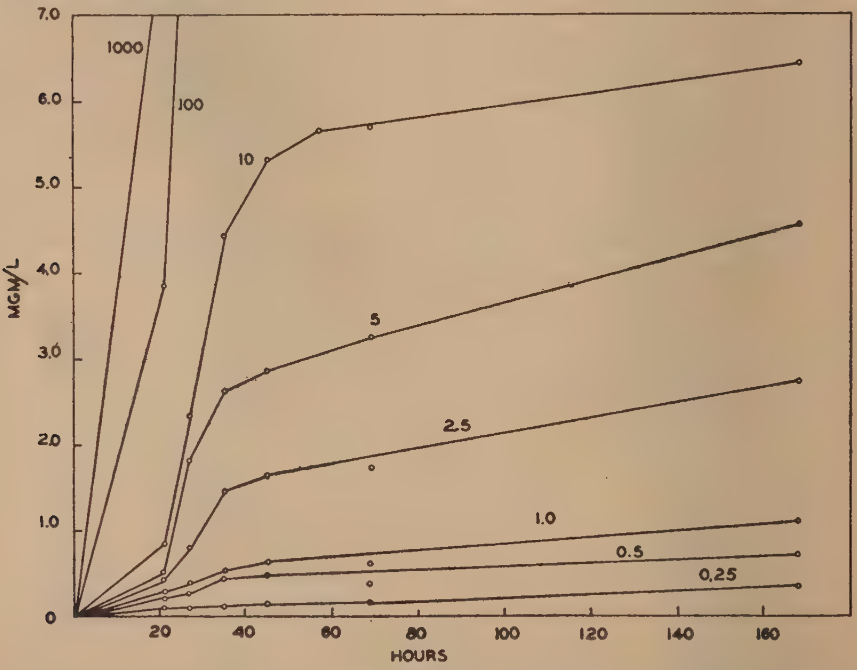


FIG. 1. AMOUNT OF OXYGEN CONSUMED BY BACTERIA GROWING IN A MINERAL SOLUTION ENRICHED WITH 0.25 TO 1000 MGM./L. OF PEPTONE AFTER DIFFERENT PERIODS OF TIME AT 22°C.

After 50 to 65 per cent of the glucose is oxidized, the rate of oxygen consumption decreases sharply regardless of the initial concentration of glucose, although there is still plenty of dissolved oxygen left. The work of ZoBell (1940) established that the rate of oxidation of organic matter by bacteria is independent of the oxygen tension until the latter is reduced to less than 0.3 mgm./l.

After 30 days incubation no glucose could be detected in the media, which initially contained 0.1 to 10 mgm./l., although oxygen consumption indicated that only 60 to 70 per cent of the glucose had been oxidized. The explanation was found in the bacterial populations of the respective media as determined by the direct microscopic procedure. The calculated weight of bacteria in the medium and on the walls of the culture vessel approximated the weight of the rest of the glucose. In other words, in dilute solutions approximately 60 to 70 per cent of the glucose is oxidized in 3 to 30 days at 22°C. and 30 to 40 per cent is converted into bacterial protoplasm. According to Rahn (1932) when the initial concentration of fermentable substance is low, considerably less CO_2 or alcohol is produced than should be expected, probably due to the utilization of a comparatively large amount of fermentable material for cell construction in a poor medium. Waksman and Carey (1935b) reported that during the storage of sea water, bacteria oxidize around 60 per cent of the organic matter and convert 40 per cent of it to bacterial protoplasm.

With prolonged incubation (60 to 120 days) oxygen consumption continues at a very slow rate. This is attributed to the oxidation of cell substance although experimental proof is lacking due to the small concentrations of reactants and the difficulties involved in estimating the bacterial populations. The decrease in the number of viable bacteria as indicated by plate counts suggests that some of the cells are dying and undergoing decomposition. This process can be accelerated by heating the old cultures to 50 or 60°C. for ten minutes.

TABLE 2

Per cent of glucose oxidized by bacteria in water treated with different concentrations of glucose based upon oxygen consumption after different periods of incubation at 22°C.

PERIOD OF INCUBATION	INITIAL CONCENTRATION OF GLUCOSE IN MGM./L.						
	0.10	0.25	0.50	1.0	2.0	5.0	10
days	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
2	56.8	44.9	33.6	27.2	12.7	5.6	5.5
3	60.1	64.1	39.2	33.6	34.4	19.4	12.8
4	67.3	65.8	52.4	42.0	39.2	27.8	14.7
5		71.2	64.9	50.1	42.6	40.3	29.2
7	68.0	69.9	57.8	60.7	51.2	44.7	34.6
10	74.8	64.1	58.5	57.0	53.8	52.7	39.3
16		72.3	56.1		58.4	61.2	46.7
20	69.5	71.2	61.7	61.6	61.5	63.2	57.9
30	66.8	71.2	67.2	60.7	65.5	60.4	63.8

With low concentrations of substrate the experimental error is relatively high.

Besides glucose and peptone, it has been demonstrated that low concentrations of glycerol, ethanol, lactate, succinate, asparagine and starch are quantitatively oxidized by bacteria occurring in sea water. The per cent of different concentrations ranging from 0.25 to 10 mgm./l. of each compound which was oxidized after 16 days incubation at 22°C. is summarized in table 3. The per cent of each compound oxidized was calculated from the amount of oxygen consumed by the bacteria in the media and the amount of oxygen which would be required to oxidize the compound completely to carbon dioxide and water. As with glucose, it was found that from 50 to 70 per cent of each compound tried was oxidized within 16 days. No significant differences were noted in the degree of oxidation of the different compounds probably because mixed cultures were used, but differences were found in the rate at which the different compounds were oxidized. Determinations made after 2, 5 and 10 days showed that ethanol, lactate, succinate, glycerol and glucose were utilized most rapidly in the order listed, while starch and asparagine were utilized more slowly.

As little as 0.1 mgm./l. of the compounds listed in table 3 was sufficient to initiate the multiplication of marine bacteria, and apparently this concentration was quantitatively utilized but with such low concentrations the experimental error is high.

TABLE 3

Per cent of different organic compounds oxidized by bacteria in water as determined by oxygen consumption after 16 days incubation at 22°C.

ORGANIC SUBSTRATE	INITIAL CONCENTRATION OF ORGANIC SUBSTRATE IN MGM./L.					
	0.25	0.50	1.0	2.5	5.0	10.0
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Glucose	68.1	67.1	60.0	62.7	57.9	36.2
Succinate	89.3	83.7	81.0	85.7	71.6	40.6
Glycerol	67.6	58.9	71.3	73.0	49.9	
Starch	58.7	62.4	55.1	56.3	51.2	31.6
Lactate	78.6	84.7	67.1	63.6	64.4	
Ethanol	79.1		63.5	62.5	56.7	
Asparagine	64.3	58.2	47.2	42.8	45.1	

EXPERIMENTS WITH PURE CULTURES

Suspensions of pure cultures of marine bacteria in organic-matter-free water were used to inoculate sterile glass-stoppered bottles which were then filled with sterile oxygenated organic-matter-free water. Eight bottles inoculated with each culture were filled with water containing 5 mgm./l. of glucose and eight were filled with water containing no glucose. Duplicates of each were analyzed for dissolved oxygen at the beginning of the experiment and the remaining bottles were placed in a sterile water bath. Oxygen consumption was determined on duplicates after 5, 10 and 15 days incubation at 22°C. Table 4 shows the amount of oxygen which was consumed in 15 days in the water with and without glucose.

It will be observed that every culture consumed significantly more oxygen in water enriched with 5 mgm./l. of glucose than in water to which no glucose was added. The data in table 4 are representative of the results obtained with 70 different cultures of marine bacteria. Not only do all of the cultures oxidize glucose; they attack concentrations as low as 5 mgm./l., and some of them quantitatively utilize this amount of glucose in 15 days. Incidentally, most of these cultures do not produce acid in glucose media and according to standard methods of the Committee on Bacteriological Technic of the S. A. B. (1940), they would be described as non-glucose-fermenters. They may not produce any gas and no acid except carbonic (which is too poorly dissociated to alter perceptibly the pH of the medium) but oxygen consumption provides incontrovertible evidence that they oxidize glucose.

An extension of the experiments with lower concentrations of glucose revealed that as little as 0.1 mgm./l. promoted the multiplication and respiration of all ten pure cultures of marine bacteria which were used as inocula. Although there were marked differences in the rate of utilization, some of the cultures quantitatively oxidized 0.1 to 5 mgm./l. of glucose in 30 days at 22°C.

It has been suggested that marine bacteria coming from an environment which is notoriously low in organic matter may be specially adapted to utilize low concentrations of organic nutrients. Therefore a few well-known cultures which are ordinarily found associated with organic matter were used to inoculate dilute glucose solutions in glass-stoppered bottles. *Escherichia coli*, *Staphylococcus citreus*, *Bacillus megatherium*, *Proteus vulgaris* and *Lactobacillus lactis* were all found to consume oxygen in glucose solutions as dilute as 0.1 mgm./l. Plate counts showed that the bacteria had multiplied, although none of the cultures caused turbidity in solutions containing less than 10 mgm./l. of glucose.

TABLE 4

Oxygen consumed by pure cultures of marine bacteria in sea water with and without the addition of glucose, after 15 days at 22°C.

CULTURE	GLUCOSE CONTENT		CULTURE	GLUCOSE CONTENT	
	None	5 mgm./l.		None	5 mgm./l.
	mgm./l.	*mgm./l.*		*mgm./l.*	*mgm./l.*
None	0.03	0.01	524	0.78	3.18
502	0.26	0.65	530	0.55	2.71
505	0.18	3.24	533	0.54	1.57
510	0.19	0.78	541	0.34	2.10
511	0.34	2.29	542	0.73	2.06
513	0.00	1.52	545	0.40	1.76
514	0.09	0.54	546	0.32	1.45
516	0.30	1.70	547	0.77	2.67
517	0.17	1.30	549	0.62	1.93
518	0.12	3.82	552	0.65	2.88
520	0.51	2.91	553	0.39	3.08
521	0.32	3.10	555	0.27	2.30
522	0.18	3.03	556	0.26	1.32

Using turbidity as a criterion of growth Bigger (1937) noted that coliform bacteria failed to "grow" in concentrations of peptone lower than 1:5000 (200 mgm./l.), but in later work in which plate counts were employed to detect growth (Bigger and Nelson, 1941), coliform bacteria were observed to multiply in glass re-distilled water which contained no more than one part of nutrient in 2,500,000 parts of water (0.4 mgm./l.). Incidentally Bigger and Nelson believed that the coliform bacteria obtained their energy autotrophically from the oxidation of minute traces of ammonium absorbed from the atmosphere, but regardless of whether the coliform bacteria utilized ammonium or peptone, the fact remains that their energy requirements were satisfied by very low concentrations. According to Heukelekian and Heller (1940) *E. coli* failed to grow when the concentration of organic nutrients was less than 0.5 to 2.5 mgm./l. unless glass beads were added to increase the solid surface.

DISCUSSION

There is no reason to believe that 0.1 mgm./l. is the lowest concentration of food which will provide for the multiplication and respiration of bacteria although lower initial concentrations have not been tried experimentally due to the difficulties of preparing mineral solutions containing lower concentrations and testing for their utilization. However, since 0.1 to 0.25 mgm./l. of food is quantitatively utilized, concentrations considerably lower must be utilized. Any single molecule of food which comes into contact with the bacterial cell may be utilized. If the molecules reach the cell faster than they are needed to maintain basal metabolism, some of the food may be converted into bacterial protoplasm. When very low (less than 10 mgm./l.) the concentration of food seems to be of importance only in so far as it influences the rate of the reaction. The rate of utilization is almost directly proportional to the concentration of food although when the concentration is very low, proportionately more of it seems to be converted into bacterial protoplasm and less is oxidized.

Anything which tends to bring the food into contact with bacterial cells will accelerate its utilization. Increasing the concentration of food or increasing the size of the inoculum accelerates the utilization of the food. When the concentration of food is very low, solid surfaces such as sand, glass beads or the walls of the culture receptacle which adsorb food and bacteria promote the multiplication of bacteria and the oxidation of organic matter. This explains why bacteria often multiply faster in water samples stored in small receptacles which present much more adsorbing surface per unit of water than in large receptacles (Whipple, 1901, ZoBell and Anderson, 1936, Lloyd, 1937). This view is substantiated by the fact that neither water volumes nor solid surfaces influence the rate of bacterial activity in water rich in organic nutrients or in water containing only dissolved nutrients such as glucose, glycerol or lactate, for example, which are not readily concentrated on solid surfaces by adsorption. The observations of the senior author that the colloidal organic matter occurring in lake water or sea water is concentrated by adsorption on glass have been confirmed by the work of Stark *et al.* (1938), Heulelekian and Heller (1940) and Harvey (1941). In the experiments of Bigger and Nelson (1941) referred to above, they found that ignited talc, asbestos, kaolin, silica, unglazed porcelain, kieselguhr, permutit and other inert solids rendered dilute nutrient solutions growth-promoting.

Much information on the factors which influence the metabolism of microorganisms could be gained by observing them in dilute nutrient solutions approximating the concentrations found in their native habitats. In dilute nutrient solutions the organisms grow more slowly, and it may be more difficult to test for end products, but the results would probably be more representative of the behavior of the microorganisms in their native habitats. Moreover, in dilute nutrient solutions there is little likelihood of the accumulation of end products influencing the activity of enzymes or possibly preventing the regeneration of enzymes (Rahn, 1932).

According to Hastings (1923) *Streptococcus lactis* and allied microorganisms behaved quite differently in dilute nutrient solutions than in the artificial nutrient solutions ordinarily used in the laboratory. In a recent personal communication Professor Hastings comments, "It has always seemed to me that we tend to grow microorganisms in the laboratory under conditions which are vastly different from those existing in nature, and that therefore the viability and vitality of the organisms as we study them may be quite different from those in nature."

SUMMARY

1. Plate counts reveal that marine heterotrophic bacteria multiply in mineral solutions containing only 0.1 mgm./l. of peptone or glucose but the media are not clouded unless the concentration of initial organic nutrient is 10 to 100 mgm./l.

2. The rate of multiplication and oxygen consumption in solutions containing less than 10 mgm./l. is more or less directly proportional to the concentration of the substrate.

3. Concentrations of glucose, glycerol, ethanol, lactate, succinate, starch and asparagine ranging from 0.25 to 5 mgm./l. were quantitatively utilized by bacteria in 16 to 30 days at 22°C. From 60 to 70 per cent of the organic substrate is oxidized and 30 to 40 per cent is converted into bacterial protoplasm.

4. Using oxygen consumption as a criterion, it was found that 70 different pure cultures of marine bacteria utilize glucose although very few of them produce acid in standard methods media. The cultures attack concentrations of glucose as low as 0.1 mgm./l.

5. *Escherichia coli*, *Staphylococcus citreus*, *Bacillus megatherium*, *Proteus vulgaris* and *Lactobacillus lactis* multiply and consume oxygen in glucose solutions as dilute as 0.1 mgm./l. Lower concentrations of nutrients were not tried due to experimental difficulties.

REFERENCES

BENECKE, W. 1933 Bakteriologie des Meeres. Abderhalden's Handb. biol. Arbeitsmethoden, **9**, 404, 717–872.

BIGGER, J. W. 1937 The growth of coliform bacilli in water. J. Path. Bact., **44**, 167–211.

BIGGER, J. W., AND NELSON, J. H. 1941 The growth of coliform bacilli in distilled water. J. Path. Bact., **53**, 189–206.

BUTTERFIELD, C. T. 1929 Experimental studies of natural purification in polluted waters. III. A note on the relation between food concentration in liquid media and bacterial growth. U. S. Pub. Health Repts., **44**, 2865–2872.

Committee of Bacteriological Technic. 1940 Manual of Methods for the Pure Culture Study of Bacteria. Biotech Publications. Geneva, N. Y.

FRIEDLEIN, F. 1928 Der quantitative Verwendungsstoffwechsel des "Paratyphus" B Bazillus, des Bacterium coli und des Bacillus pyocyaneus. Z. Biochem., **194**, 273–289.

GEIGER-HUBER, M. 1934 Über den Einfluss der Konzentration des Atmungsmaterials die Atmungsgeschwindigkeit von Pflanzen. Jahrb. wiss. Botan., **81**, 1–34.

HARVEY, H. W. 1941 On changes taking place in sea water during storage. J. Marine Biol. Assoc. United Kingdom, **25**, 225–233.

HASTINGS, E. G. 1923 Comparative resistance of bacteria from native habitats and from artificial cultures. J. Infectious Diseases, **33**, 526–530.

HENRICI, A. T. 1939 The distribution of bacteria in lakes. Am. Assoc. Advanc. Sci., **10**, 39–64.

HEUKELEKIAN, H., AND HELLER, A. 1940 Relation between food concentration and surface for bacterial growth. J. Bact., **40**, 547–558.

HUCKER, G. J., AND CARPENTER, D. C. 1927 The relation of hydrolytic products of proteins to bacterial growth. J. Infectious Diseases, **40**, 485–496.

LLOYD, B. 1937 Bacteria in stored water. J. Roy. Tech. Coll. (Glasgow) **4**, 173–177.

PENFOLD, W. J., AND NORRIS, D. 1912 The relation of concentration of food supply to the generation time of bacteria. J. Hyg., **12**, 527–531.

RAHN, O. 1932 Physiology of Bacteria. P. Blakiston's and Son, Philadelphia, 438 pp.

STARK, W. H., STADLER, JANICE, AND MCCOY, ELIZABETH. 1938 Some factors affecting the bacterial population of freshwater lakes. J. Bact., **36**, 653–654.

WAKSMAN, S. A., AND CAREY, C. L. 1935 Decomposition of organic matter in sea water by bacteria. I. Bacterial multiplication in stored sea water. J. Bact., **29**, 531–543.

WAKSMAN, S. A., AND CAREY, C. L. 1935 Decomposition of organic matter in sea water by bacteria. II. Influence of addition of organic substances upon bacterial activity. J. Bact., **29**, 545–561.

WHIPPLE, G. C. 1901 Changes that take place in the bacterial contents of waters during transportation. Tech. Quart., **14**, 21–29.

ZOBELL, C. E., AND ANDERSON, D. Q. 1936 Observations on the multiplication of bacteria in different volumes of stored sea water and the influence of oxygen tension and solid surfaces. Biol. Bull., **71**, 324–342.

ZOBELL, C. E. 1940 Some factors which influence oxygen consumption by bacteria in lake water. Biol. Bull., **78**, 388–402.

ZOBELL, C. E. 1941 Studies on marine bacteria. I. The cultural requirements of heterotrophic aerobes. J. Marine Research, **4**, 42–76.

ZOBELL, C. E., AND GRANT, C. W. 1942 Bacterial activity in dilute nutrient solutions. Science, **96**, 189.

Contribution No. 200

CHLOROPHYLL-CONTENT OF MARINE PLANKTON[1]

By

HERBERT W. GRAHAM
Mills College, Oakland, California
and
Scripps Institution of Oceanography, University of California,
La Jolla, California

INTRODUCTION

With the recent progress of oceanography and the accumulation of data regarding the biological and chemical conditions of ocean water many oceanographers have become interested in correlations which relate to organic production and chemical cycles in the sea (Redfield, 1934; Seiwell, 1935; Harvey, et al, 1935; Fleming, 1940; Riley, 1941b). Since such studies require an accurate knowledge of conditions in the sea it is of prime importance to have some method for measuring the potential synthetic capacity of the sea which can be used in survey work as well as at fixed stations.

One of the best methods for determining organic production in the sea is by measuring the oxygen production of a sample *in situ* (Nielsen, 1937). However, this method is not practicable for general oceanographic work. The time-honored method of counting the number of plant cells is not satisfactory. First, it is a slow and tedious procedure. Second, the results must be expressed in numbers of cells when the size of the cells in different species or organisms is very different. Furthermore, silk bolting cloth which is usually used for collecting the samples on board ship is not a reliable filter. The centrifuge method, also, has fallen into disrepute (Nielsen, 1933). In an attempt to find some way of determining the synthetic potentialities of serial samples Harvey (1934) made acetone extracts of plankton and used the intensity of color as an index of the amount of phytoplankton present by comparison with an artificial standard. Since Harvey used silk bolting cloth for filtration his method contains all the errors of the net methods. Furthermore, the "plant pigments" extracted by Harvey include pigments such as carotenoids which are extractable from many animals as well as plants (Gillam, et al, 1939) so that the "Harvey Unit" is not strictly a phytoplankton unit.

---

[1] Contribution from the Scripps Institution of Oceanography, New Series No. 200.

Reprint from SEARS FOUND. JOURN. MAR. RES.

Better substances to measure are the chlorophylls. Not only are they strictly plant pigments but they are the enzymes directly responsible for organic production. A measure of the chlorophyll-content of the water is a measure of the photosynthetic capacity of the water. The chlorophyll of plankton has been determined by a few workers. Kreps and Verjbinskaya (1930) determined the chlorophyll-content of net plankton from the Barents Sea. Kozminski (1938) measured the chlorophyll of plankton in Wisconsin Lakes obtained with a Sharpless centrifuge. Riley (1938a) used the Harvey Standard as a chlorophyll standard after calibrating with a pure chlorophyll solution. He used paper for filtration. Gillam, et al (1939) included chlorophyll in their biochemical analysis of net plankton from the North Sea. Krey (1939) collected plankton from the region of Kiel with a "cella" filter and measured its chlorophyll-content spectrophotometrically.

METHOD

A practical method to be used in survey work for determining the chlorophyll-content of plankton has the following requirements:

1. Use of small quantities of water.
2. Rapid filtration.
3. Removal of at least 95 per cent of the phytoplankton.
4. Use of small amount of solvent.
5. Rapid reading of chlorophyll concentration.
6. Use of portable equipment.

The present investigations were carried out on the surface plankton off the pier of the Scripps Institution of Oceanography at La Jolla, California during the summers of 1941 and 1942. Four liters of water were collected in a bucket, poured into a glass bottle, removed to the laboratory, and filtered. After testing many kinds of filters, fine chemical filter paper was selected as the most suitable. With the plankton in this locality this filter retained more than 95 per cent of the microplankton and yet permitted rapid filtration. With the use of a little suction four liters were filtered in 15 minutes. Rapid filtration is possible only if the entire surface of the paper is used. This was effected by supporting the paper on the wire gauze of a Seitz bacteriological filter.

The filter paper with contained plankton was placed in 10 ml of 80 per cent acetone in the dark for one-half hour. The resulting extract was passed through a fine sintered glass filter and examined in a Bausch and Lomb visual spectrophotometer. Absorption of light was measured at wave length of maximum absorption in the red, namely, at 668 mu with collimator slit width of 0.4 mm. Using the absorption coefficient 82.04 determined by MacKinney (1941) for chlorophyll *a*, the concentration of chlorophyll was computed and expressed as milligrams per cubic meter of seawater.[1] Concurrently with the determinations of chlorophyll, phytoplankton counts were made on samples settled in graduated cylinders. The results are presented in Table I and Figure 43.

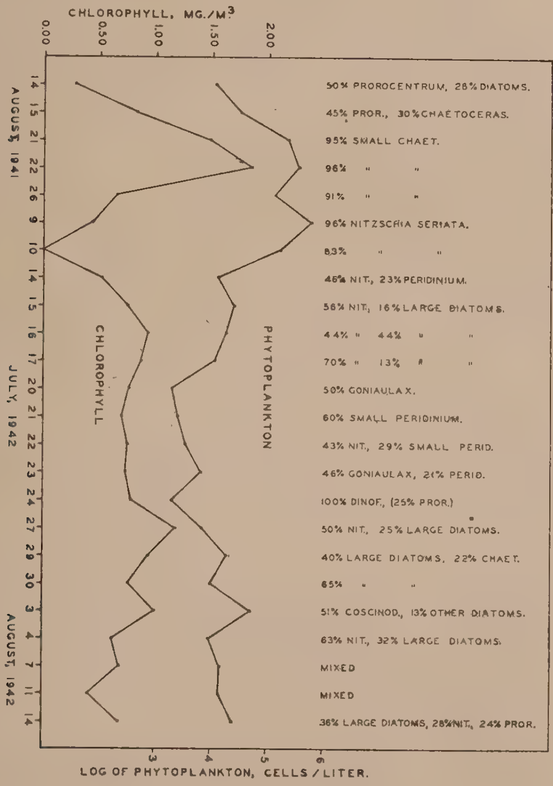


Figure 43.

---

[1] The absorption maximum of cholorophyll *b* in the red is not far from that of chlorophyll *a* and its concentration in plankton is probably negligible. However, chlorofucine has a maximum somewhat apart, at 627 mu in ether (Strain and Manning, 1942). How abundant this pigment is, is not known but an examination of the raw extract of phytoplankton suggests that it does not occur in large quantity in marine plankton.

This method of determination of plankton chlorophyll satisfies all the requirements listed above except the use of portable equipment. Work is now in progress on the construction of a portable monochrometer especially designed for chlorophyll determinations on board ship.

RESULTS AND DISCUSSION

The concentration of chlorophyll in the plankton at the Scripps pier varied from almost none on July 10, 1942 to 1.86 mg/m^3 on August 22, 1941 (Table I and Figure 43). The average value for the twenty-four determinations made throughout the summers of 1941 and 1942 was 0.77 mg. These values are within the range of values found by other workers (Table II).

The phytoplankton counts made at the same time as the chlorophyll determinations varied from 2,000 to 573,000 cells per liter. During the periods of investigation the composition of the plankton was changing continually. The dominant members of the phyto-

TABLE I—Comparison of Total Cell Count, Chlorophyll-content, and Composition of Phytoplankton at La Jolla, California

Date	*Total Cell Count*	*Chlorophyll mg/m^3*	*Composition of Phytoplankton*
1941			
Aug. 14	11,600	0.26	50% *Prorocentrum* spp.; 28% *diatoms*
Aug. 15	30,450	0.80	45% *Prorocentrum* spp.; 30% *Chaetoceras* spp
Aug. 21	219,600	1.46	95% small *Chaetoceras* spp.
Aug. 22	343,160	1.86	96% small *Chaetoceras* spp.
Aug. 26	132,000	0.65	91% small *Chaetoceras* spp.
1942			
July 9	573,000	0.46	96% *Nitzschia seriata*
July 10	168,000	trace	83% *N. seriata*
July 14	13,000	0.53	46% *N. seriata;* 23% *Peridinium* spp.
July 15	25,000	0.75	56% *N. seriata;* 16% large diatoms
July 16	18,000	0.92	44% *N. seriata;* 44% large diatoms
July 17	11,500	0.87	70% *N. seriata;* 13% large diatoms
July 20	2,000	0.76	50% *Goniaulax* spp.
July 21	2,500	0.70	60% small *Peridinium* spp.
July 22	3,500	0.75	43% *N. seriata;* 29% small *Peridinium* spp.
July 23	6,500	0.74	46% *Goniaulax* spp.; 21% *Peridinium* spp.
July 24	2,000	0.78	100% dinoflagellates (25% *Prorocentrum* spp.)
July 27	7,000	1.20	50% *N. seriata;* 25% large diatoms
July 29	18,500	0.93	40% large diatoms; 22% *Chaetoceras* spp.
July 30	10,000	0.76	65% large diatoms
Aug. 3	51,000	1.01	50% *Coscinodiscus* sp.; 13% other diatoms
Aug. 4	9,500	0.62	63% *N. seriata;* 32% large diatoms
Aug. 7	15,000	0.68	Mixed
Aug. 11	14,000	0.40	Mixed
Aug. 14	25.000	0.68	36% large diatoms; 28% *N. seriata;* 24% *Prorocentrum* spp.

TABLE II—Comparison of Chlorophyll Determinations in Different Regions by Different Workers. Values in mg/m³

Worker	*Region*	*Depth*	*Filter*	*Mean*	*Maximum*
Kreps and Verjbinskaya, 1930	Barents Sea	upper 25 meters	net	0.02	0.12
Kozminski, 1938	Wisconsin lakes	various	centrifuge	10.0[1]	386.0
Gillam, et al, 1939	North Sea	upper 60 meters	net	0.01	0.135
Krey, 1939	Kiel	upper 30 meters	"cella"	2.00	5.00
Riley, 1938b	Tortugas	surface	paper	0.33	—
Riley, 1939a	Western North Atlantic	surface	paper	1.2	3.6
Riley, 1940	Linsley Pond	upper 13 meters	paper	14.7	40.0
Riley, 1941a	Long Island Sound	surface	paper	17.44	62.0
Riley, 1941b	Georges Bank	surface	paper	5.6	27.2
Graham, 1942	La Jolla	surface	paper	0.77	1.86

[1] Approximate.

plankton community are indicated in Table I and Figure 43 for each day of observation.

The amount of chlorophyll in the plankton should bear a fairly definite relation to the total amount of phytoplankton protoplasm. It can be assumed that the relation between chlorophyll-content and protoplasm may vary widely between species or even according to age or other physiological conditions within a species, so that quantitative determinations of the green pigments cannot be used as an exact measure of plant protoplasm present. However, phytoplankton cell counts are not good indices of the relative degree of biosynthesis in a given sample or region because of the extreme differences in cell size and skeleton weight among the various species. Therefore, chlorophyll assays should afford a more reliable measure of the potential organic synthesis in any given region than the numbers of phytoplankton cells.

The few data available on the chlorophyll-content of phytoplankton species emphasize the differences in species of different sizes. Harvey (1934) estimated that one plant pigment unit was equivalent to 6950–7300 cells of *Rhizosolenia alata* but to only 224 cells of *Biddulphia regia*. According to Krey (1939) one gamma of chlorophyll is equivalent to 286,000 cells of *Chaetoceras gracile.* Our own data indicate that one gamma is equivalent to 150,000–190,000 cells of *C. Van Heurckii.* The minute species of *Nitzschia* contain much less chlorophyll per cell. Pace (1941) estimated that one gamma of chlorophyll was equivalent to 1,840,000 cells of *N. closterium* while our own data show that one gamma is equivalent to 1,250,000 cells of *N. seriata.* It is probable that much smaller numbers of the larger diatoms and dinoflagellates are necessary to supply the equivalent of chlorophyll although data concerning this are not yet available.

Considering the great difference in chlorophyll-content of different kinds of plankton we should not expect a series of phytoplankton counts to vary in accordance with variations in total chlorophyll-content of the plankton. A glance at Figure 43 indicates some general correspondence in the variation of the two factors. A closer examination, however, shows that there is no direct relationship between chlorophyll-content and numbers of cells. If the composition of the phytoplankton is taken into consideration, however, most of the discrepancies can be accounted for.

When the observations were started August 14, 1941 the phytoplankton population was 50 per cent *Prorocentrum*. At that time the chlorophyll-content and the cell count were comparatively low. In a few records the cell counts and chlorophyll-contents rise and fall together until there is an abrupt change in the composition of the plankton from predominately small *Chaetoceras* species to *Nitzschia seriata*, a very small needle-shaped species. While the percentage of *Nitzschia* is high the chlorophyll content is low. As the percentage of *Nitzschia* decreases the amount of chlorophyll increases in proportion to the cell count.

In the next few measurements a medium amount of chlorophyll is maintained by relatively low numbers but, significantly, the phytoplankton community is represented more by dinoflagellates on these days. During the later observations, excepting on July 29, there was a good correspondence between chlorophyll-content and cell count. Mixed phytoplankton communities obtained at those times.

At inshore stations a certain amount of algal detritus probably contributes some chlorophyll. What percentage of the total this may reach has not been determined. It is highly desirable that the present studies be extended to offshore stations when conditions allow.

When portable equipment has been perfected for routine determinations of chlorophyll such work should give values very useful for general oceanographic work. Although it is not intended that chlorophyll determinations should entirely replace cell counts, quantitative chlorophyll data should be more easily interpreted in terms of the chemical conditions of the water than the data regarding the numbers of phytoplankton cells or the amount of acetone soluble pigments of total microplankton. Meanwhile, continued parallel studies of total chlorophyll and of relative numbers of various species of phytoplankton should provide useful information for various branches of oceanographic study.

The writer is indebted to the Director, Dr. H. U. Sverdrup, of the Scripps Institution of Oceanography and to other members of the

staff, particularly Dr. D. L. Fox who graciously offered the facilities of his laboratory and gave much helpful advice during the progress of these investigations.

REFERENCES

Fleming, R. H.
1940. Composition of plankton and units for reporting populations and production. Proc. 6th Pac. Sci. Cong. 535–540.
Gillam, A. E., Redi, M. S., and Wimpenny, R. S.
1939. The seasonal variation in biological composition of certain plankton samples from the North Sea. Jour. Exper. Biol. *16:* 71–88.
Harvey, H. W.
1934. Measurement of phytoplankton population. Jour. Mar. Biol. Ass. United Kingd. *19:* 761–773.
Harvey, H. W., Cooper, L. H. N., Lebour, M. V., and Russell, F. S.
1935. Plankton production and its control. Jour. Mar. Biol. Ass. United Kingd. *20:* 407–441.
Kozminski, Z.
1938. Amount and distribution of the chlorophyll in some lakes of northeastern Wisconsin. Trans. Wisc. Acad. Sci. *31:* 411–438.
Kreps, E. and Verjbinskaya, N.
1930. Seasonal changes in the Barents Sea. Jour. du Conseil. *5:* 329–345.
Krey, J.
1939. Die Bestimmung des Chlorophylls in Meerwasser-Schöpproben. Jour. du Conseil. *14:* 201–209.
Mackinney, G.
1941. Absorption of light by chlorophyll solutions. Jour. Biol. Chem. *140:* 315–322.
Nielsen, E. S.
1933. Ueber quantitative Untersuchung von marinem Plankton mit Utermöhls umgekehrten Mikroskop. Jour. du Conseil. *8:* 2.
1937. The annual amount of organic matter produced by the phytoplankton in the sound off Helsingör. Medd. Komm. f. Danmarks Fiskeri-og Havundersögelser. Serie: Plankton, Bd. III, Nr. 3, 38 pp.
Pace, Nello
1941. Pigments of the marine diatom *Nitzschia closterium.* Jour. Biol. Chem. *140:* 483–489.
Redfield, A. C.
1934. On the proportions of organic derivatives in sea water and their relation to the composition of plankton. James Johnstone Memorial Volume (Univ. Liverpool Press) 176–202.
Riley, G. A.
1938a. The measurement of phytoplankton. Int. Rev. Ges. Hydrobiol. Hydrogr. *36:* 371–373.
1938b. Plankton studies, I. A preliminary investigation of the plankton of the Tortugas region. Jour. Mar. Res. *1:* 335–352.

1939a. Plankton studies. II. The western North Atlantic, May–June 1939. Jour. Mar. Res. *2:* 145–162.

1939b. Correlations in aquatic ecology. Jour. Mar. Res. *2:* 56–73.

1940. Limnological studies in Connecticut. Part III. The plankton of Linsley Pond. Ecol. Monogr. *10:* 279–306.

1941a. Plankton studies. III. Long Island Sound. Bull. Bing. Ocean. Coll. *7* (3): 1–89.

1941b. Plankton studies. IV. Georges Bank. Bull. Bing. Ocean. Coll. *7* (4): 1–73.

SEIWELL, H. R.

1935. The annual organic production and nutrient phosphorus requirement in the tropical western North Atlantic. Jour. du Conseil. *10:* 20–32.

STRAIN, H. H. AND MANNING, W. M.

1942. Chlorofucine (Chlorophyll γ), a green pigment of diatoms and brown algae. Jour. Biol. Chem. *144:* 625–636.

Contribution No. 201

Reprinted from the
ANNALS OF THE NEW YORK ACADEMY OF SCIENCES
Vol. XLIV, Art. 1. Pages 19–40
May 29, 1943

SOURCES OF ATMOSPHERIC HEAT AND MOISTURE OVER THE NORTH PACIFIC AND NORTH ATLANTIC OCEANS*

BY WOODROW C. JACOBS†
Scripps Institution of Oceanography, University of California, La Jolla, California

INTRODUCTION

The present paper is a summary of results of an investigation concerning the exchange of heat and water vapor between the sea surface and atmosphere over the North Pacific and North Atlantic oceans. The theoretical aspects of the investigation, together with the derivations of equations, have already been presented in a previous paper[2] and will not be elaborated here.

Briefly, the method consisted of developing an equation which would express the rate of evaporation as a function of the difference in vapor pressure between sea surface and atmosphere, and of the wind movement within the turbulent layer, using available climatic data over the oceans. The original Sverdrup equation[10] was used, assuming an atmosphere in unstable or neutral equilibrium, the constants of which were derived empirically by comparing the mean annual evaporation over the oceans as computed by such a formula with similar values obtained through the use of the energy equations. When using mean climatic data over the oceans, it was found that the evaporation could be determined by means of the following equation,

$$E = 0.143\,(e_w - e_a)\,U_a \qquad \text{mm/24-hours} \qquad (1),$$

where e_w is the vapor pressure at the sea surface (millibars), e_a the vapor pressure at 6 meters height and U_a the wind speed (meters per second) at 6 meters.

It was shown further that the amount of sensible heat exchanged between sea and atmosphere could be computed by means of the Bowen formula[1] in the form:

$$Q_c = 0.65\left(\frac{t_w - t_a}{e_w - e_a}\right) L_t\,E, \qquad (p = \text{const.} = 1000\text{ mb}) \qquad (2),$$

and in which t_w and t_a are sea surface and air temperatures, respectively,

---

*Contributions from the Scripps Institution, New Series, No. 201.
†Present address, U. S. Weather Bureau, Washington, D. C.

L_t is the latent heat of vaporization of water at temperature t, and E is expressed in grams or centimeters.

Through the use of equations (1) and (2), computations of the mean seasonal values for evaporation, heat exchange and total energy exchange were made for each five-degree square in the North Pacific and North Atlantic. The charts constructed from the data showing the total energy exchange between sea and atmosphere are of particular interest to the field of oceanography since the loss or gain of energy from the sea surface through either conduction or evaporation is entirely in the form of heat. On the other hand, the charts showing the mean seasonal and annual values for E and Q_c are of interest to the meteorologist, who is concerned not only with the amount of heat added to or subtracted from the lower layers of the atmosphere, but also with the amount of latent energy in the form of water vapor which is rendered available for the various meteorological processes. The previously mentioned paper[2] emphasized the oceanographic aspects of the investigation; the present paper is more concerned with the meteorological significance of the results.

SEASONAL AND REGIONAL VARIATIONS IN THE RATE OF EVAPORATION OVER THE NORTH PACIFIC AND NORTH ATLANTIC

The distribution of values for the mean daily evaporation within each 5-degree square over the North Pacific and North Atlantic oceans, as computed by means of equation (1), during winter (December, January, February) and during summer (June, July, August) is illustrated in FIGURES 1 and 2. These charts show that evaporation over the oceans is considerably greater during winter than during the summer months, particularly at higher latitudes and over the western portions. They also show that, in general, the regions of greatest evaporation are located along the eastern coasts of Asia and North America over the Kuroshio and Gulf Stream. Secondary areas of high evaporation appear about the southern margins of the North Atlantic and North Pacific semi-permanent high-pressure cells, i.e., in the northern part of the trade-wind region. Thus, in general, it is shown that the regions of greatest evaporation are those within which the northerly transport of surface waters is greatest, and over areas that are subjected during winter to frequent invasions by cold, dry continental air masses from the interiors of Asia or North America. An examination of the mean fields of motion over the Far East and the eastern coasts of North America shows that the pre-

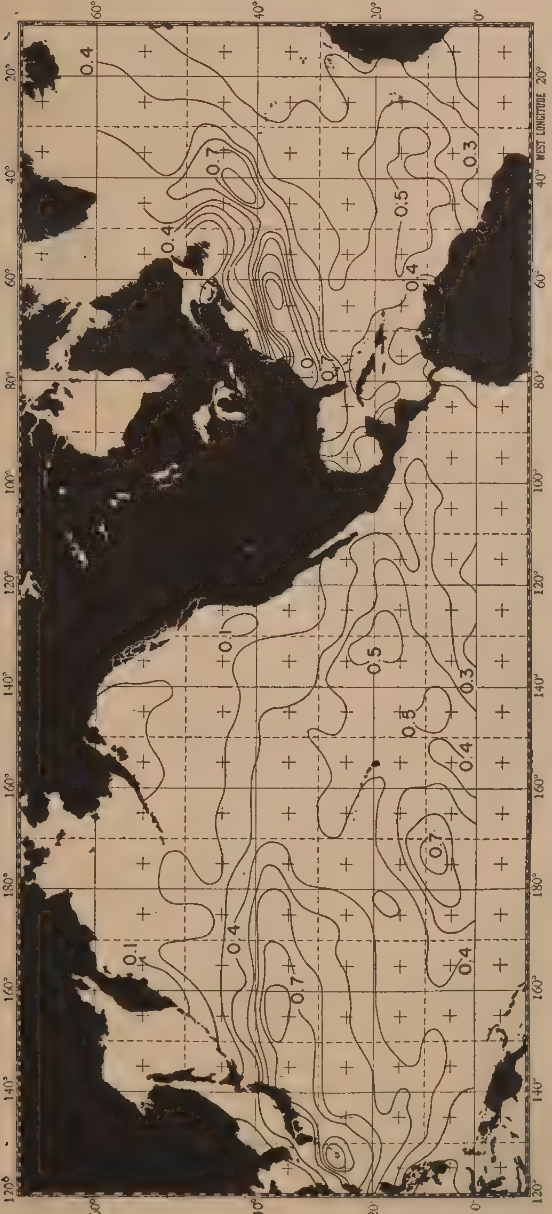

60°
40°
20°
0°
20°
WEST LONGITUDE
0.4
0.7
0.4
0.5
0.3
0.4
1.0
0.7
0.1
0.5
0.3
0.5
0.4
0.7
0.4
0.4
0.1
0.7

FIGURE 1.

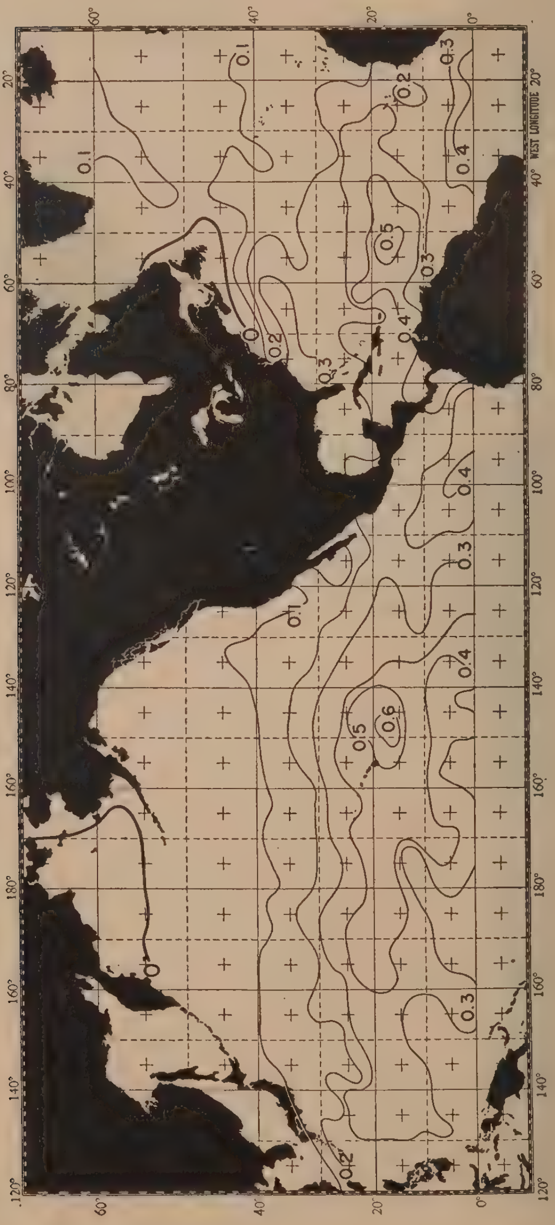

60°
40°
20°
0°
20°
40°
60°
80°
100°
120°
140°
160°
180°
160°
140°
120°
WEST LONGITUDE 20°
0.1
0.2
0.3
0.4
0.5
0.6

FIGURE 2.

vailing air-mass types over the western sides of the oceans during winter are of continental origin, i.e., cPk or cAk air masses.*

The regions of least evaporation are those of southerly-flowing ocean currents along the extreme northwest coasts and along the eastern sides of the oceans. These are regions which, except in the northwest portions, are most frequently under the influence of air masses with a relatively long maritime history.

The great amount of evaporation within the trade-wind belts appears to be associated with the dry, descending air currents accompanying the semi-permanent fields of high pressure. A relatively low evaporation over the equator in the eastern North Pacific during spring appears to be associated with the northward-flowing ocean currents which in this region cross the equator resulting in an influx of cold water from higher latitudes in the Southern Hemisphere.

In summer the evaporation reaches its lowest value over most of the North Pacific and North Atlantic except within the eastern equatorial regions of both oceans, where the maximum values are reached during this season. A slight net condensation is computed for this season within the North Pacific north of latitude 55° and west of 160° W, and in the North Atlantic waters immediately surrounding Labrador.

In the North Atlantic Ocèan, the center of the tropical or trade-wind area of maximum evaporation remains nearly stationary during all seasons except autumn, when it quite largely disappears, with its mean position located between latitudes 10° and 20° N, and approximately one-third the distance from the coast of South America to West Africa. In the North Pacific Ocean, on the other hand, this tropical area of high evaporation is considerably better developed and the position of its center varies with the seasons. In winter the maximum evaporation within this area is 0.70 cm/day and the center is located between 5° and 10° N latitude and 170° and 175° W longitude. In spring the maximum increases slightly to 0.72 cm/day and the center is displaced northeastward to the area between 10° and 15° N latitude and 150° and 155° W longitude. In summer the maximum evaporation in this region decreases to 0.62 cm/day and the center is displaced farther northeastward to the area between 15° and 20° N latitude and 145° and 150° W longitude. In the autumn, however, the maximum evaporation within this area falls to its lowest value, 0.56 cm/day, and the now less well-defined center is displaced southward to the general region on the equator between 0° and 5° N latitude, and 135° and 150° W longitude.

---

* *Note:* cPk = Polar continental air colder than the surface over which it is passing, cAk = Arctic air colder than the surface. For description of the air-mass types see Petterssen, Weather Analysis and Forecasting, pp. 166–204 (1941).

This clockwise migration of the tropical center of high evaporation in the North Pacific appears to correspond to the similar movement of the center of the North Pacific high-pressure field.

The average seasonal values of evaporation for the various latitude ranges are given in FIGURE 3. These curves illustrate very clearly the winter maximum of evaporation in nearly all latitudes and the much lower summer evaporation within the higher latitudes. They also show that the seasonal variation in evaporation at low latitudes is much smaller than in the higher latitudes; that evaporation is higher during autumn than during spring in the middle and high latitudes but that, except at the equator, the reverse is true at the lower latitudes. Within the latitude range 0° to 5° N in the North Atlantic, the evaporation values are lowest during winter but in the North Pacific they are lowest during spring.

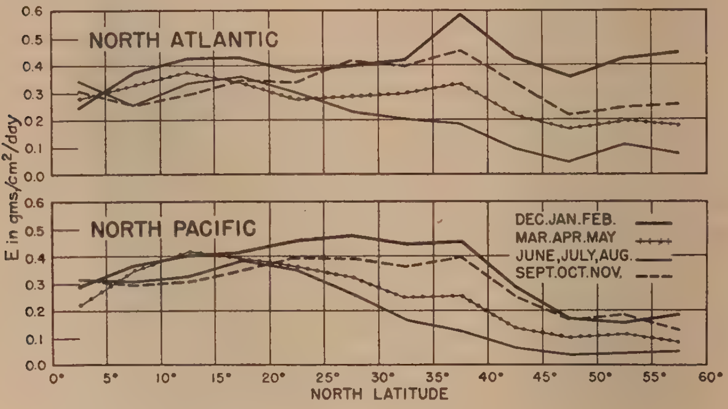


FIGURE 3.

THE TOTAL EVAPORATION OVER THE NORTH PACIFIC AND NORTH ATLANTIC

As shown in FIGURE 4, the latitudinal distribution of values representing the total volume of water evaporated from the oceans (ΣE) is quite different from the distribution of values representing the evaporation per unit area of sea surface (E). This is due to the unequal distribution of areas of sea surface over the Northern Hemisphere (see TABLE 1). These curves show that the greatest quantity of water is evaporated in the lower latitudes during all seasons and that the quantity is greatest in winter, least in summer and somewhat greater during autumn than during spring.

As shown by the data in TABLE 2, the seasonal variations in ΣE are greater in the North Atlantic than in the North Pacific, the percentage difference between the winter and summer evaporation being 13.8 per cent of the total annual evaporation in the North Atlantic and only 9.6 per cent of the total annual evaporation in the North Pacific. Although

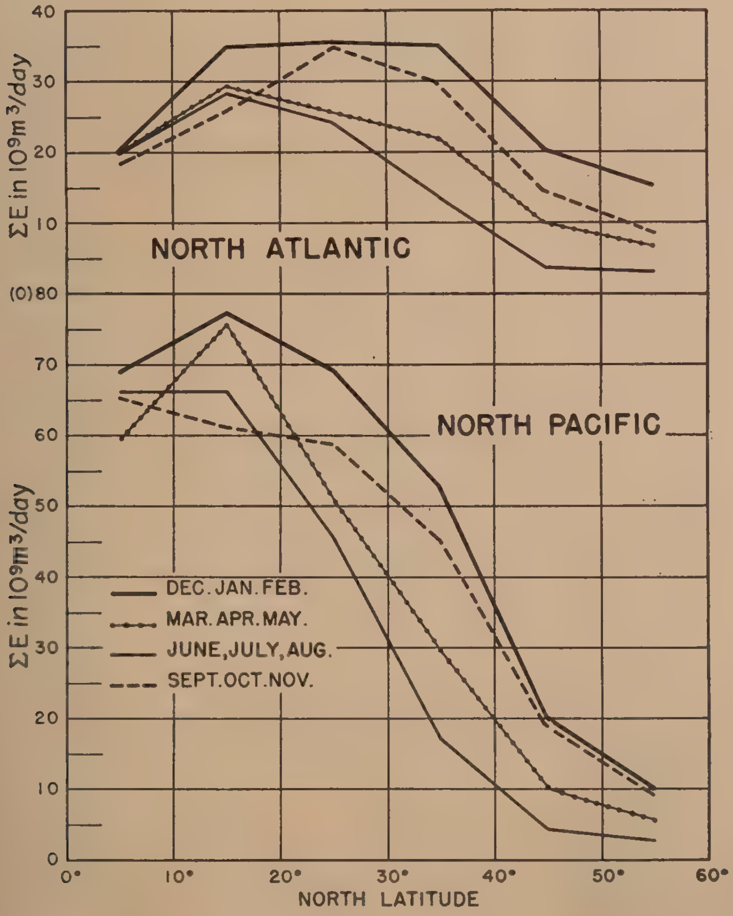


FIGURE 4.

TABLE 1

DISTRIBUTION OF AREAS IN THE NORTH PACIFIC AND NORTH ATLANTIC BY LATITUDE ZONES AND AS A WHOLE*

	North Pacific				North Atlantic			
North Latitude range (°)	Area 1000 km^2	Per cent of total (North Pacific plus North Atlantic)	Per cent of total (North plus South Pacific)	Per cent of North Pacific	Area 1000 km^2	Per cent of total (North Pacific plus North Atlantic)	Per cent of total (North plus South Atlantic)	Per cent of North Atlantic
0–10	21,246	76.4	12.0	26.0	6,545	23.6	7.2	14.3
10–20	18,656	69.5	10.5	22.9	8,181	30.5	9.0	17.9
20–30	14,922	61.9	8.5	18.3	9,180	38.1	10.1	20.0
30–40	11,707	62.6	6.6	14.3	7,000	37.4	7.7	15.3
40–50	8,685	62.6	4.8	10.6	5,181	37.4	5.7	11.3
50–60	5,773	62.0	3.3	7.1	3,545	38.0	3.9	7.7
(60–70)†	(643)	(17.6)	(0.4)	(0.8)	(3,000)	(82.4)	(3.3)	(6.5)
(>70)	—	(0.0)	—	—	(3,182)	(100.0)	(3.5)	(7.0)
	Total 81,632	% of total 64.1	% of total 46.1	Total 100.0	Total 45,814	% of total 35.9	% of total 50.4	Total 100.0

*Computed from data by Schott.[7,8]

†Areas in parentheses are not included in the energy computations.

the area of the North Pacific between the equator and latitude 60° N ($80,989 \times 10^3$ km^2) is 67.1 per cent of the total area ($120,621 \times 10^3$ km^2) of both oceans between the same parallels, it accounts for only 66.5 per cent of the total volume of water evaporated from both areas, due to the slightly greater rate of evaporation in the North Atlantic. The total volume of water evaporated from the North Pacific during the course of the year averages $90,232.4 \times 10^9$ m^3, which is equivalent to an evaporation rate of 111.4 cm/year. The total volume of water evaporated from the North Atlantic during the year amounts to $45,490.0 \times 10^9$ m^3, which is equivalent to a rate of 114.8 cm/year. The quantity for both oceans is $135,722.4 \times 10^9$ m^3/year, equivalent to a mean depth of 112.5 cm/year. The higher rate of evaporation in the North Atlantic appears to be due largely to the smaller amount of sensible heat given off to the atmosphere rather than to any significant excess of energy available at the surface. But an analysis of cloud charts for the Northern Hemisphere[9] indicates that the mean annual cloudiness between the equator

TABLE 2
MEANS OF THE TOTAL DAILY EVAPORATION (ΣE) OVER THE NORTH PACIFIC AND NORTH ATLANTIC

Area	Season	Mean ΣE	ΣE for both oceans	Mean annual ΣE
		10^6 m^3/day	Per cent	Per cent
North Pacific	Winter	297,019	64.8	30.0
	Spring	231,834	67.3	23.5
	Summer	202,054	68.5	20.4
	Autumn	257,942	66.2	26.1
	Year	247,212	66.5	100.0
North Atlantic	Winter	161,314	35.2	32.4
	Spring	112,637	32.7	22.6
	Summer	92,940	31.5	18.6
	Autumn	131,627	33.8	26.4
	Year	124,630	33.5	100.0
Both Oceans	Winter	458,333	100.0	30.8
	Spring	344,471	100.0	23.2
	Summer	294,994	100.0	19.8
	Autumn	389,569	100.0	26.2
	Year	371,842	100.0	100.0

Total Annual Evaporation:—
North Pacific
= $90,232.4 \times 10^9$ m^3/year: equivalent to mean depth of 111.4 cm/year.
North Atlantic
= $45,490.0 \times 10^9$ m^3/year: equivalent to mean depth of 114.8 cm/year.
Both oceans
= $135,722.4 \times 10^9$ m^3/year: equivalent to mean depth of 112.5 cm/year.

and latitude 60° N may be somewhat greater in the North Pacific than in the North Atlantic. Rough estimates by scalage indicate the average annual cloudiness over the North Pacific is about 60 per cent; over the North Atlantic about 55 per cent. If these latter figures are valid, they may partly account for the greater evaporation rate in the North Atlantic.

By means of reductions from pan observations at sea, Wüst (1920; 1936) has derived values for the mean annual evaporation at various latitudes in the several oceans. His figures for the North Pacific and North Atlantic, however, extend only from the equator to latitude 40° N. By taking the mean of Wüst's values for three adjacent 5-degree parallels as being representative of the mean evaporation within each 10-degree latitude zone, his figures show an evaporation of 127.5 cm/year in the North Atlantic and 118.3 cm/year in the North Pacific. The present calculations for the same latitude range (0° to 40° N) give 123.0 cm/year for the North Atlantic and 124.5 cm/year for the North Pacific. The mean value for this same area in both oceans is 124.0 cm/year, thus about 2 per cent greater than Wüst's average (121.2 cm/year) for the two oceans.

Wüst gives 93 cm/year as the average evaporation for all oceans. This low value is due to including evaporation in the Southern Hemisphere where the values appear to be lower at most latitudes than in the Northern Hemisphere. This latter condition is particularly true over the extensive areas of sea surface at high latitudes in the Southern Hemisphere. For example, his value for the latitude range (45° to 50° S) within the Atlantic, Pacific and Indian Oceans is only 43 cm/year.

The seasonal ratios between the total daily evaporation in the North Pacific and North Atlantic are given in TABLE 3. These data show the ratio $\Sigma E_{\text{Pacific}}/\Sigma E_{\text{Atlantic}}$ to be greatest during summer, least during winter and greater during spring than autumn. The reasons for these variations appear to be greater contrasts between the winter and summer air-mass types over the North Atlantic than is the case in the North Pacific. More explicitly, the winter air masses over the North Atlantic are drier and colder relative to the summer air masses than are those of the North Pacific, which is not surprising when one considers the smaller area of the North Atlantic and the corresponding closer proximity of the continental land masses with their wide seasonal variations in temperature and humidity.

It was brought out in the previous paper that the locations of the principal frontal zones over the oceans as given by Petterssen[6] correspond quite closely with the zones of maximum energy exchange. The trade-wind area of maximum evaporation in the North Pacific lies im-

TABLE 3
SEASONAL RATIOS BETWEEN TOTAL DAILY EVAPORATION IN THE NORTH PACIFIC AND ATLANTIC OCEANS

Season	Ratio $\frac{\Sigma E \text{ (N. Pacific)}}{\Sigma E \text{ (N. Atlantic)}}$
Winter	1.84
Spring	2.06
Summer	2.17
Autumn	1.96
Year	1.98

mediately east and south of his mean winter position for the mid-Pacific Polar Front in the Northern Hemisphere, with the principal axis of the frontal zone parallel to the major axis of the zone of maximum evaporation. On the other hand, the maximum zones of winter evaporation in the western parts of the two oceans lie largely to the west and north of Petterssen's positions for the polar fronts in winter over these areas. The lines or zones of frontogenesis (cyclogenesis), however, will coincide more nearly with the zones of maximum temperature gradient immediately off the coasts of East Asia and eastern North America. Thus, in the western portions of the oceans, the principal frontogenetic areas will be located along the eastern coasts of the two continents northwestward from the mean positions of the polar fronts themselves. This is due to the fact that the zones of frontogenesis will tend to move with the air currents toward the axis of outflow and will assume a mean position near this axis or trough line in the mean flow. It is found that in both oceans the zones of frontogenesis (cyclogenesis) will lie in every case to the northwestward of a zone of maximum evaporation and that the major axes of the zones of maximum evaporation will parallel the principal axes of the frontal zones.

It is to be remarked that the mean seasonal data shown in FIGURES 1 and 2 are by no means representative of instantaneous conditions over the oceans. It is expected that non-periodic variations in the rates of evaporation over the oceans will occur and that the areas of maximum and minimum evaporation will also vary in both intensity and position. Thus over the Kuroshio and Gulf Stream, at the times of outbreaks of cold cAk and cPk air from the continents in winter, the evaporation values may occasionally be extremely high. Conversely, during periods in winter when the polar fronts are located near the coasts and the air masses over the neighboring ocean areas are of southerly origin (mE or mT masses),* the evaporation in these areas must reach low values. Also, along the west coasts of the continents, the evaporation in winter may reach high values at the times of outbreak of dry air from the interior, as during the periods when the so-called "Santa Ana" winds blow from the east or northeast along the coasts of Southern California. Just as there are important non-periodic variations in the positions of the principal centers of action in the atmosphere, the polar fronts, etc., with the resulting time or regional variations in humidity, wind, temperature, cloudiness, etc., so must there be corresponding changes in the evaporation over the oceans. It is expected that the regional variations in evaporation (or heat exchange) will be most pronounced during periods with a low zonal index and that the evaporation over the oceans will be most uniform during periods with a high zonal index.[5]

If secular variations in the amount of solar energy received at the surface of the earth occur, it is to be expected that similar secular variations in evaporation and heat exchange will also take place. But it is not suggested that such variations must be proportional, since the effects of increased humidity, extent of cloudiness, etc., may be important.

VARIATIONS IN THE AMOUNT OF SENSIBLE HEAT EXCHANGED BETWEEN SEA AND ATMOSPHERE

The distribution of values representing the amount of sensible heat exchanged between sea and atmosphere over the North Pacific and North Atlantic during winter and summer are shown in FIGURES 5 and 6. The isolines for Q_c show, roughly, the same configuration as shown for E, these values being at their maximum in winter and along the western sides of the oceans. One very important difference is shown. No tropical areas of maximum heat exchange exist within the trade-wind region to correspond to the areas of maximum evaporation that appear to be associated with the North Pacific and Azores high-pressure fields.

During winter there are three areas of maximum heat exchange in the North Pacific, the principal area occurring within the Kuroshio between 35° and 40° N, and 150° and 155° E where Q_c averages 246 g.cal.cm^2/day, with secondary areas northeast of Formosa (Taiwan) and in the northern portion of the Sea of Japan. The area northeast of Formosa coincides with the area of maximum winter evaporation in the North Pacific (see FIGURE 1). The maximum value for Q_c in the North Atlantic (274 g.cal.cm^2/day) occurs within the Gulf Stream between latitudes 35° and

---

*mE = maritime equatorial air; mT = maritime tropical air.

40° N, about 700 miles east of the Virginia capes, with a secondary belt of high values for Q_c extending north and south along the 40° W meridian between latitudes 40° and 55° N. The North Pacific values for Q_c during winter are positive for all regions except for several small areas near the equator. In the North Atlantic, however, the charts show that Q_c is negative during winter for approximately one-third the area of that ocean.

In summer the values for Q_c become insignificant nearly everywhere in the North Pacific except in the southeastern equatorial regions where values as high as 40 g.cal.cm^2/day are determined, and are negative everywhere north of latitude 40°, off the California coast and, surprisingly, also off the Asiatic coasts south to the Philippine Islands. In the North Atlantic during summer, Q_c is negative everywhere except within a narrow zone along the eastern coasts of North America extending northeastward from Florida to latitude 40° N, and within the Gulf of Mexico and Caribbean Sea. The average seasonal values of Q_c for the different latitude ranges are given in FIGURE 7. These curves illustrate very clearly the winter maximum in the heat exchange at nearly all latitudes and the pronounced maxima for Q_c above latitude 35° N in both oceans. During autumn, winter and spring, the values for Q_c increase generally from the equator to maxima in both oceans between 35° and 40° N, decrease to minima between latitudes 45° and 50° N, and then increase again poleward. During summer, however, the values for Q_c in both oceans generally decrease from the equator toward higher latitudes. One interesting point to note in these curves is the fact that the values for Q_c are higher in the North Pacific than in the North Atlantic at all latitudes and during all seasons except during winter for the areas north of latitude 35° N. This question will be discussed in greater detail under the section on the regional and seasonal variations in the values for the ratio between evaporation and heat exchange.

From the data so far presented it thus appears that the sea is heating the atmosphere by significant amounts only in the middle and high latitudes, along the eastern sides of the continents and principally during the winter season. In other portions of the oceans and almost everywhere during summer, the sea is actually receiving some energy from the atmosphere.

Data showing the latitudinal distribution of the mean annual values for Q_c indicate that in both oceans the maxima occur between latitudes 35° and 40° N, with low values at the equator and a secondary minimum in each ocean between latitudes 45° and 50° N. It has previously been pointed out that the mean annual rate of evaporation in the North

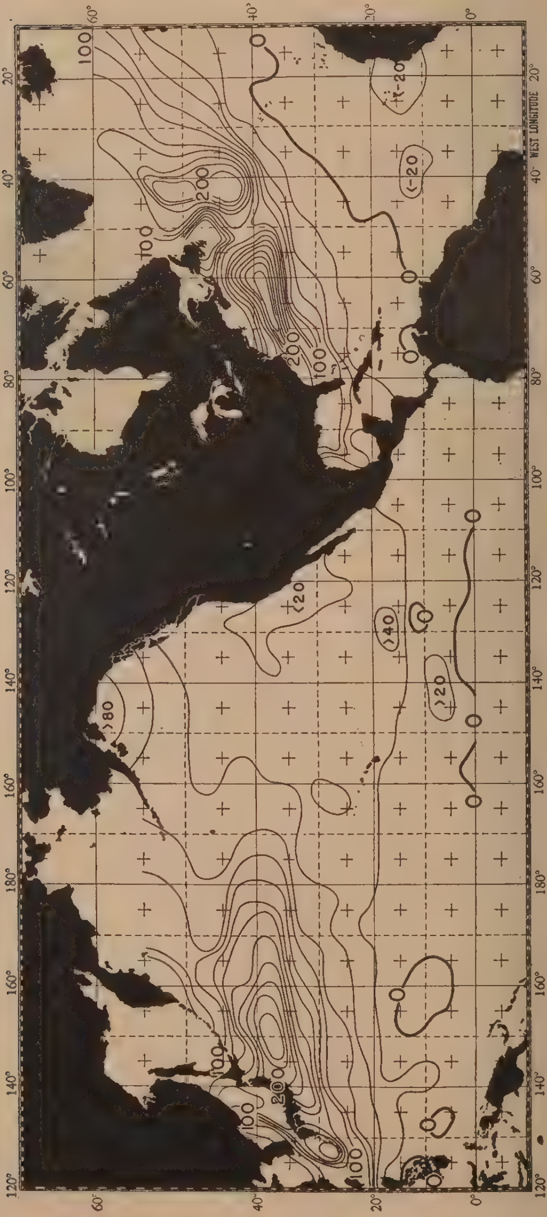

60°
40°
20°
0°
20°
40°
60°
80°
100°
120°
140°
160°
180°
160°
140°
120°
WEST LONGITUDE
100
200
0
<-20
<20
>40
>20
>80

FIGURE 5.

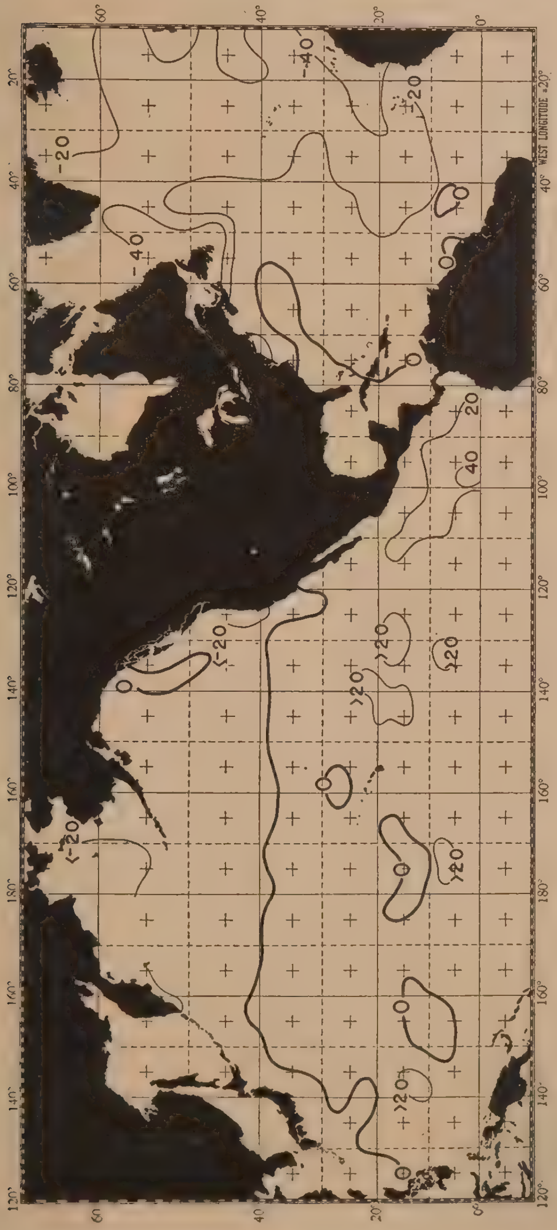

60°
40°
20°
0°
20°
40°
60°
80°
100°
120°
140°
160°
180°
160°
140°
120°
WEST LONGITUDE
-20
-40
-40
-20
0
20
40
<-20
>20
>20
>20
>20
0
<-20
0
>20
0
>20
0

FIGURE 6.

Pacific is highest between latitudes 20° and 25° N and in the North Atlantic between latitudes 35° and 40° N, with a secondary maximum

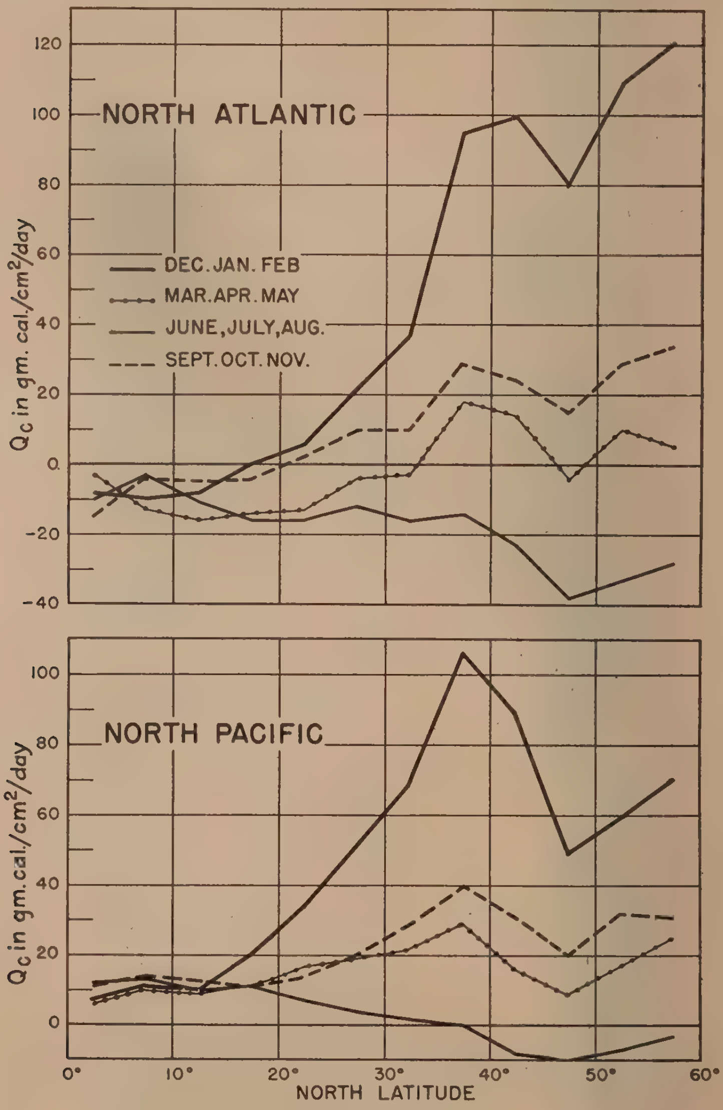


FIGURE 7.

between latitudes 15° and 20° N. Comparing the two sets of data, it is found that the zone of maximum evaporation in the North Pacific occurs farther south than the zone of maximum heat exchange, the displacement being 15 degrees of latitude. In the North Atlantic, however, the zones of maximum evaporation and maximum heat exchange coincide except that there is no secondary zone of maximum heat exchange within the latitude range 15° and 20° N to correspond to the secondary zone of maximum evaporation in this area. These conditions indicate that over the oceans as a whole the atmosphere is being heated most rapidly in the higher latitudes but that it is receiving moisture principally in the middle and lower latitudes.

VARIATIONS IN THE RATIO (R) BETWEEN THE AMOUNT OF HEAT GIVEN OFF TO THE ATMOSPHERE AS SENSIBLE HEAT (Q_c) AND THE AMOUNT OF HEAT USED FOR EVAPORATION (Q_e)

In previous investigations of evaporation from the oceans, the ratio between the amount of heat given off to the atmosphere by convection and the amount of heat used for evaporation was assumed a constant for lack of better information. For example, in their computations of the mean annual evaporation at various latitudes over the oceans, Mosby[4] has assumed R constant at 0.10; McEwen[3] at 0.20. However, the present computations show that this ratio is a highly variable quantity, both seasonally and with respect to the regional distribution. The mean seasonal values for R arranged by latitudes are shown in FIGURE 8. These curves illustrate that at all latitudes, the values for R are higher in the North Pacific than in the North Atlantic; that they are highest at all latitudes in both oceans during winter, lowest during summer, and about the same in spring as in autumn. During all seasons except summer, the values for R in both oceans increase from low values at the equator to maxima at high latitudes. During summer, the values are highest near the equator and decrease to minima in both oceans between latitudes 45° and 50° N, and then increase toward the poles. Thus (except during summer), while evaporation within the equatorial and tropical regions is high, the exchange of heat between sea and atmosphere is relatively small and, conversely, although evaporation at high latitudes is small, the exchange of heat between sea and atmosphere is relatively great. Similarly, it must be concluded that a greater amount of the available energy at the sea surface in the North Pacific is used in heating the atmosphere directly than is the case in the North Atlantic where a greater proportion is used in the evaporation process.

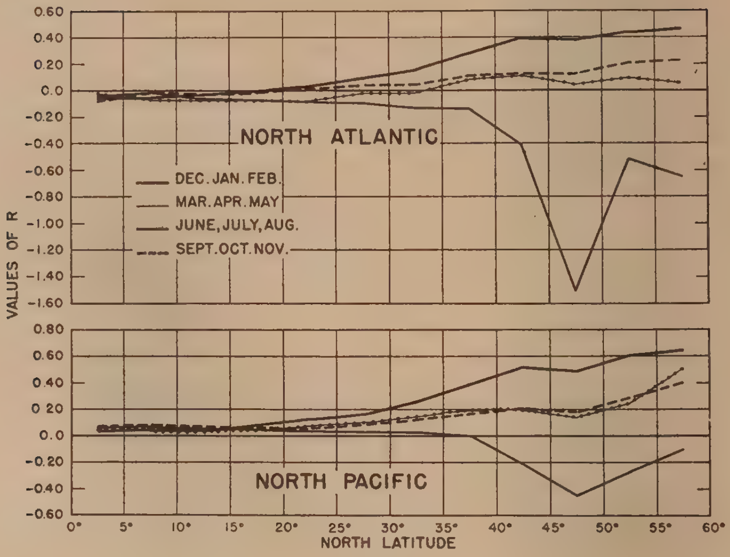


FIGURE 8.

The principal reason for the higher values for R in the North Pacific appear quite obvious. Since the ratio, Q_c/Q_e, is given by

$$R = 0.65 \frac{(t_w - t_a)}{(e_w - e_a)} \frac{p}{1000} \qquad (3),$$

it follows that either vapor pressures over the North Pacific must be lower than over the North Atlantic or the quantities $(t_w - t_a)$ must be greater. The data show no significant differences between vapor pressures over the two oceans but an analysis of charts showing the differences between sea and air temperatures over the North Pacific and North Atlantic (U. S. Weather Bureau, 1938) shows very clearly that the positive temperature differences (sea minus air) are greater over the North Pacific as a whole than over the North Atlantic. The curves showing the latitudinal distribution of the mean annual values for R indicate, however, that the differences (R_{Pacific} minus R_{Atlantic}) are very nearly constant for all latitudes at about 0.10 or 0.11 (see TABLE 4). It is difficult to account for this constancy of differences.

It is to be noted that all computations of evaporation, heat exchange and total energy exchange have been made from data accumulated over the two oceans at or near Greenwich mean noon. Thus, most of the observations in the North Atlantic have been made during daylight hours

TABLE 4

LATITUDE VARIATION OF DIFFERENCE BETWEEN *R* OF THE PACIFIC AND OF THE ATLANTIC

North Latitude Range (°)	**R* (Pacific) Minus *R* (Atlantic)	
0–5	0.10	
5–10	0.11	(0.106)
10–15	0.10	
15–20	0.10	
20–25	0.11	(0.106)
25–30	0.08	(0.11)
30–35	0.14	
35–40	0.10	
40–45	0.11	
45–50	0.12	
50–55	0.10	
(55–60)†	(0.21)	
Mean	0.11	

*Mean of the ratio between the average annual values for Q_e and Q_c.
†Not considered in the mean.

between 0600 and 1200, local mean time, while those in the North Pacific have been made largely during night hours between 2000 and 0600, local mean time. Although the diurnal variations in evaporation or heat exchange over the oceans can be assumed to be small (Sverdrup, 1940), the values computed from the uncorrected observational data obtained on shipboard might conceivably lead to significant differences and thus might account for the constancy of differences between the values for *R* in the North Pacific and North Atlantic. The hourly observational data necessary for computations of the hourly evaporation or heat exchange over the oceans are not generally available. There is a long series of observations for the area around the East Indies (between latitudes 12° S and 10° N; longitudes 112° and 134° E) obtained by the *Snellius Expedition.*[13] The results of the computations of *R* on the basis of the bihourly values of sea-surface temperature, air temperature, vapor pressure and wind speed for areas farther than 100 km. from the coasts, are shown in FIGURE 9. The curve showing the diurnal variation of *R* is rather irregular but with a decided minimum at midnight and a well-defined maximum at about 1000. Actually, the variations are of small magnitude since the vertical scale on FIGURE 9 is greatly exaggerated. Between the hours of 0600 and 1200, the mean value for *R* proves to be 0.070; between the hours of 2000 and 0600, 0.052. Thus it appears that

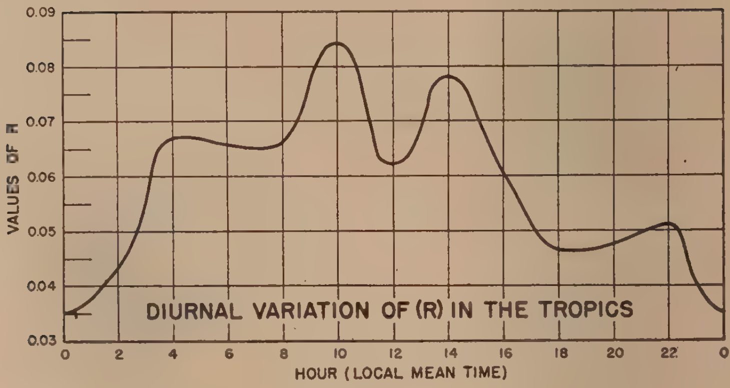


FIGURE 9.

the constancy of differences between the computed values for R in the North Pacific and North Atlantic at the various latitudes can hardly be explained on the basis of the diurnal variabilities in the quantities Q_c and Q_e, and the reasons for the constancy of differences are not apparent at the present time.

Before closing the discussion of the seasonal and latitudinal variations in R, it should be brought out that the computed values at very high latitudes may be questionable. This is true for two reasons: (1) the areas involved in the computations of R for high latitudes are rather small; and (2) equation (3) is very sensitive to small, and otherwise insignificant, observational errors (or the dropping of decimals in the means) at very low values for the quantities $(e_w - e_a)$ or $(t_w - t_a)$. Thus, at high latitudes (above 55° N) where Q_c and Q_e are very small, these small errors may be significant when computing R.

CONCLUSION

The present paper has attempted merely to present a few of the more important conclusions to be drawn from the computations of mean seasonal evaporation and heat exchange over the North Pacific and North Atlantic. The investigation is being continued at the Scripps Institution with some emphasis being placed upon the short-period and non-periodic variations in these quantities over the oceans. It is expected that these results, together with the complete series of charts showing the seasonal and annual values for E, Q_c and Q_a (the total energy exchanged between sea and atmosphere $= Q_e + Q_c$) and more detailed analyses of the data, will be presented in a later publication.[2]

ACKNOWLEDGMENT

The writer wishes gratefully to acknowledge the assistance and advice given by Dr. H. U. Sverdrup of the Scripps Institution of Oceanography, during all stages of the investigation.

DISCUSSION OF THE PAPER

Prof. C. F. Brooks (*Blue Hill Observatory, Milton, Mass.*):

I wonder if the systematic error in the determination of sea-surface temperature by bucket—the method used in the observations summarized and mapped by the Weather Bureau—may not be involved to a significant degree in the difference in computed evaporation between the Atlantic and Pacific in the diurnal range of *R* and in the general total. In 5 observational periods totalling 9 weeks at sea, both in the Atlantic and Pacific, in all seasons of the year and over a range of latitude from 7° to 60°, I have checked the accuracy of sea-temperature, air-temperature and humidity observations made by ships' personnel on American, Canadian, German and Norwegian steamships—with some surprising results. Only the sea-temperature checks have been published (in part).* The systematic error of a determination of sea-surface temperature is of the order of $-\frac{1}{2}°$ C., the observed temperature being below the true temperature. In the Gulf Stream off Cape Hatteras in winter the average error is about $-3°$ C.

The error at night is greater than by day, by perhaps ½° C. on the general average, but increasing in proportion to the evaporation. At night: (1) the wet bucket is colder, not being sunned; (2) the making of a catch takes more time; (3) the reading of the thermometer takes longer—either bucket or thermometer must be taken to a light. Thus, the computed evaporation during the night in the Pacific should be lower than during the day in the Atlantic. Furthermore, the error of the bucket reading will be proportional to the evaporation. Therefore, the ratio *R* will differ by essentially the same amount in all latitudes, as found by Mr. Jacobs.

The diurnal course of *R* is apparently also influenced by this diurnal course of error.

The total annual computed evaporation is probably below the true evaporation. Perhaps this explains why Mr. Jacobs' values were somewhat lower than previous computations.

Observations of air and wet-bulb temperatures are subject also to appreciable error. Air temperatures reported at sea in the daytime are almost universally too high—owing to the ship's influence. Wet-bulb temperatures are also too high, and for added reasons. The wet-bulb covering is usually saline. (The wet-bulb is sometimes too high by being immersed in a bottle of water!) The result is that the vapor pressures computed from observations at sea are too high.

Reply by Mr. W. C. Jacobs:

It was realized in the beginning that the observational data over the oceans would present serious errors. However, the final evaporation equation obtained was prepared to fit just these sort of climatic data. Such a procedure is valid if the errors of observation can be handled in statistical fashion—the computations show that such is the case, therefore we cease to have any further interest in their magnitude (see ref. 2 for full discussion).

Prof. Brooks suggests that the evaporation computed by the above method may prove to be too low. While this might be the case when applying the theoretical Sverdrup equation to raw ocean data, the assigning of correction factors based on comparisons between annual evaporation amounts computed on the basis of the several energy equations and those computed on the basis of the Sverdrup equation has raised the amounts by approximately 30 per cent. Contrary to the statement of Prof. Brooks, the computed values are not lower than those of previous investigators. For example, Wüst's annual values are 2 per cent lower for both

---

*C. F. Brooks, Mon. Weather Rev., **54**: 241, 1926; Journal Wash. Acad. Sci., **18**: 529, 1928.

oceans (3 per cent lower in the case of the North Pacific; no difference in the case of the North Atlantic). Similarly, the computed evaporation is slightly higher than that given by McEwen and by Schmidt but slightly lower than that of Mosby, the latter of whom admits that his values constitute an upper limit and are probably too high to accurately represent average conditions. The lower evaporation rate in the North Pacific is also in agreement with previous investigations.

FIGURE 9 was prepared to show the small magnitude of the differences in the computed values for E and Q_o resulting from time differences in observations and was not intended to show the true diurnal variability of R. Correcting the data for diurnal effects (or errors), then, would actually result in lower values for R (higher evaporation) in the North Atlantic and higher values for R (lower evaporation) in the North Pacific, thus increasing the constant range of differences shown in TABLE 4 instead of decreasing them as is suggested by Prof. Brooks. Actually, however, the total difference (0.018) is too small to be considered of great significance.

REFERENCES

1. **Bowen, I. S.**
1926. The ratio of heat losses by conduction and by evaporation from any water surface. Phys. Rev. **27**: 779–787.

2. **Jacobs, W. C.**
1942. On the energy exchange between sea and atmosphere, Sears Found. Jour. Mar. Res. **5** (1): 37–66.

3. **McEwen, George M.**
1938. Some energy relations between the sea surface and the atmosphere. Sears Found. Jour. Mar. Res. **1** (3): 217–238.

4. **Mosby, H.**
1936. Verdunstung und Strahlung auf dem Meere. Ann. der Hydrog. u Marit. Meteor. **54**: 281–286.

5. **Namias, J., & Wexler, H.**
1942. Suggested in a personal communication to the writer.

6. **Petterssen, Sverre**
1940. Weather analysis and forecasting. 1st ed. New York. 503 p.

7. **Schott, Gerhard**
1912. Geographie des Atlantischen Ozeans. Hamburg. 330 p.

8. 1935. Geographie des Indischen und Stillen Ozeans. Hamburg. 413 p.

9. **Shaw, Sir Napier**
1936. Manual of meteorology. Vol. II. 2d edition. London. p. 146.

10. **Sverdrup, H. U.**
1937. On the evaporation from the oceans. Sears. Found. Jour. Mar. Res. **1** (1): 3–14.

11. 1940. On the annual and diurnal variation of the evaporation from the oceans. Sears. Found. Jour. Mar. Res. **3** (2); 93–104.

12. **U. S. Weather Bureau**
1938. Atlas of climatic charts of the oceans.

13. **Visser, S. W.**
1936. The Snellius-Expedition. Vol. III, Meteorological results. Leiden. 111 p.

14. **Wüst, G.**
1920. Die Verdunstung auf dem Meere. Veröff. Inst. f. Meereskunde, Berlin. N. F., A, Geogr. Naturw. Reihe, Heft 6, Oct. 1920.

15. 1936. Oberflächensalzgehalt, Verdunstung, und Niederschlag auf dem Weltmeere. *In:* Festschrift Norbert Krebs. 347–359.

Contribution No. 202

Reprinted from the
Annals of the New York Academy of Sciences
Vol. XLIV, Art. 1. Pages 81–88
May 29, 1943

ON THE RATIO BETWEEN HEAT CONDUCTION FROM THE SEA SURFACE AND HEAT USED FOR EVAPORATION*

By H. U. Sverdrup
Scripps Istitution of Oceanography, University of California, La Jolla, California

Since the evaporation from the oceans by means of the energy equation was first calculated[11] in 1915, numerous attempts have been made to use this procedure and to apply it to other problems of similar nature. If no energy flows through the solid boundaries of a body of water the energy equation takes the form $Q_a = Q_r - Q_\vartheta$, where Q_a represents the energy which is given off from the water surface to the air, Q_r is the net radiation received, that is, the difference between the incoming radiation from sun and sky and the outgoing long-wave radiation of the surface, and Q_ϑ is the heat used in changing the temperature of the water.

In the above form the energy equation simply states that the difference between energy received and used for changing the temperature must be given off to the atmosphere. In order to find the evaporation it is necessary to consider that the energy given off to the air is composed of two terms, one representing the energy used for evaporation, Q_e, and one representing that which is conducted to the air as sensible heat, Q_h. Thus, $Q_a = Q_e + Q_h$. Introducing the ratio, $R = \frac{Q_h}{Q_e}$, and writing $Q_e = LE$, where L is the latent heat of evaporation and E is the evaporation, one obtains

$$E = \frac{Q_r - Q_\vartheta}{L\,(1 + R)} \qquad (1),$$

where the units of E depend upon the units in which the other terms are measured.

Since Q_r and Q_ϑ can be obtained accurately from adequate observation, it is important also to determine R. In his computations, Schmidt assumed values of R ranging from 2.3 to 0.3. Ångström[1] pointed out that these values were probably too high and, from a consideration of the processes of heat conduction and evaporation, he concluded that on an average R was probably about 0.1. This value was used by Mosby[8] when he revised Schmidt's figures.

Meanwhile Bowen[2] had established an expression for R which is strictly correct only under the conditions considered by him. This expression, "the Bowen ratio," has been applied to atmospheric conditions, particularly by Cummings and Richardson,[6] Richardson;[9] and Cummings[4,5] who have assumed that the Bowen ratio would be exactly valid in this case as well. An analysis of the general validity of the Bowen ratio will be attempted here.

---

*Contributions from the Scripps Institution of Oceanography, New Series, No. 202.

The two fundamental equations from which Bowen starts can be written

$$Q_h = D_2 c_p \rho(\vartheta_1 - \vartheta_2)/l \qquad (2),$$

$$Q_e = D_1 L (\rho_1' - \rho_2')/l \qquad (3),$$

where D_2 is the "diffusion coefficient" for heat energy and D_1 is the diffusion coefficient of water vapor through air. These coefficients have been found to differ only by a few per cent, as predicted by the kinetic gas theory. Furthermore, c_p is the specific heat at constant pressure, ρ the density of the air, ϑ_1 and ϑ_2 the air temperature at the two faces of a space of length l, ρ_1' and ρ_2' the densities corresponding to the vapor pressures, and L is the latent heat of vaporization.

Bowen discusses three different cases, but it is sufficient here to consider case II, in which it is assumed that heat and water vapor are carried away by processes of diffusion only. On these assumptions, the ratio, Q_h/Q_e is

$$R_B = \frac{D_2}{D_1} \frac{c_p \rho}{L} \frac{\vartheta_1 - \vartheta_2}{\rho_1' - \rho_2'} \qquad (4).$$

Introducing the sufficiently accurate expression

$$\rho' = 0.621 \, \rho \frac{e}{p} \qquad (5),$$

where e is the vapor pressure and p is the atmospheric pressure; putting $\vartheta_1 = \vartheta_w$, the temperature of the water surface, $\vartheta_2 = \vartheta_a$, the temperature of the air at any height above the water, $e_1 = e_w$, the vapor pressure at the water surface, and $e_2 = e_a$, the vapor pressure in the air at the height where the temperature was measured; and using the numerical values $D_2 = 0.181 \; f(\vartheta)$, $D_1 = 0.206 \; f(\vartheta)$, $c_p = 0.241$, and $L = 585$, Bowen obtains

$$R_B = 0.58 \frac{\vartheta_w - \vartheta_a}{e_w - e_a} \frac{p}{1000} \qquad (6).$$

It is here assumed that Q_h and Q_e are independent of the height above the water surface; that is, that stationary conditions exist. Furthermore, the assumption is made that the transfer of heat energy and water vapor takes place by ordinary diffusion, in which case the corresponding coefficients are functions of temperature only. These functions are similar and the ratio D_2/D_1 is therefore independent of temperature and also independent of the distance from the water surface.

The fundamental equations from which Bowen starts apply to transfer of heat and water vapor by processes of ordinary diffusion. It might therefore appear that the computed ratio R_B is valid only when the air is at rest or in laminar motion, but, as stated by Bowen, one can expect "that heat losses by evaporation and diffusion and by conduction will follow the same laws and will be affected in the same way by convection" (turbulence). This implies that the ratio between the heat losses will be independent of the state of turbulence, or, that the ratio, R_B, will be valid regardless of the character of the air motion. Recent results suggest, however, that when examining the transfer of heat energy it may be necessary to take into account the effect of processes of radiation upon the transfer of heat and the effect of spray upon evaporation. If such processes have to be considered it can be expected that heat losses by conduction, and by evaporation and diffusion, will be affected differently by turbulence and that the Bowen ratio may have to be modified.

Let us first examine the effect of heat transfer by radiation when the air is at rest or in laminar motion. In a moist atmosphere a radiant flux of heat is directed from regions of higher to regions of lower temperature (Brunt, 1934, p. 119). This flux is dependent upon the selective emission and absorption of long-wave radiation by the water vapor, and is not present in dry air because dry air does not emit or absorb long-wave radiation. The effect of this flux of radiation can be described by means of a coefficient which Brunt calls radiative diffusivity. The radiative diffusivity, K_R, has the same dimensions (cm²/sec) as the diffusion coefficient for heat energy, D_2, and when problems of diffusion of heat energy in moist air are considered, must be added to D_2. Doing this, equation (6) is altered to

$$R = \frac{D_2 + K_R}{D_1} 0.66 \frac{\vartheta_w - \vartheta_a}{e_w - e_a} \frac{p}{1000} \qquad (7).$$

Thus, the ratio R is greater than R_B and the increase depends upon the relative magnitudes of D_2 and K_R. Unfortunately, very little is known about the value of K_R near a boundary surface. At distances above the ground greater than about 40 meters, K_R has a value, according to Brunt, of the order of 10^3cm²/sec and is thus about 10^4 times greater than D_2, but it seems certain that K_R must decrease rapidly when approaching a boundary surface. The very great value computed by

Brunt at a moderate height strongly suggests that near a boundary surface K_R may be of the same order of magnitude as D_2 (about 0.2 cm^2/sec) and that in the absence of turbulence R is many times greater than indicated by Bowen's formula. Theoretical and experimental examination of this question seems desirable.

In the presence of turbulence, neglecting radiative diffusivity, the equations for transfer by eddy diffusivity of heat energy and of energy in the form of latent heat take the form

$$Q_h = -\frac{\mu_e}{\rho} c_p \rho \left((d\vartheta/dz) + \gamma\right) \qquad (8),$$

$$Q_e = -\frac{\mu_e}{\rho} L \, d\rho'/dz \qquad (9),$$

where μ_e/ρ is the eddy diffusivity and γ is the adiabatic lapse rate, 1.0C.° per 100 meters.

In a steady state Q_h and Q_e are independent of the height above the water surface and represent the quantities indicated by the same symbols in equations (2) and (3). With the above numerical value, the equation

$$R = 0.66 \frac{p}{1000} \left(\frac{(d\vartheta/dz) + \gamma}{de/dz}\right) \qquad (10),$$

can therefore be considered valid within the turbulent layer. If this equation were valid from the very water surface, the differentials could be replaced by differences and

$$R = 0.66 \frac{p}{1000} \frac{\vartheta_w - \vartheta_a - \gamma h}{e_w - e_a} \qquad (11),$$

where h is the height at which the air temperature is measured, would be an exact equation for the ratio Q_h/Q_e.

This expression for R differs little from equation (6) if $(\vartheta_w - \vartheta_a)$ is much greater than γh. This is usually the case and the Bowen ratio, R_B, is therefore applicable, as pointed out by Bowen, in the presence of turbulence if other processes can be disregarded.

In the turbulent layer near the ground the effect of radiative diffusivity can probably be disregarded because at moderate and high wind velocities the eddy diffusivity is many times greater than the estimated radiative diffusivity, but observations of temperature and vapor pressure directly above a water or snow surface indicate the existence of a boundary layer through which the transfer of energy takes place by processes of molecular diffusion and in which radiative diffusivity may be important. This boundary layer is described by Brunt[3] (p. 262) as a time-mean phenomenon:

> "Water-vapor produced by evaporation at a liquid surface spreads outward through the laminar layer by molecular diffusion, while the air within that layer is from time to time exchanged by the action of eddies which penetrate it."

Above the boundary layer the transfer of heat and water vapor takes place by eddy diffusion as expressed by equations (8) and (9). As a first approximation it may be assumed that, near a surface which is hydrodynamically rough, the eddy diffusivity is a linear function of the height above the surface (Rossby[10]), in which

$$\mu_e/\rho = k_0 w^*(z + z_0) \qquad (12),$$

where $k_0 = 0.4$ is von Kármán's constant and w^* is the friction velocity ($w^* = \sqrt{\tau_0/\rho}$, where τ_0 is the stress of the wind). Assuming this value of the eddy diffusion above the boundary layer and introducing the ordinary diffusion coefficient in the boundary layer,[13] the upward transfer of water vapor can be represented as a function of the difference $(e_w - e_a)$ and, following a similar procedure, the heat transfer can similarly be expressed as a function of $(\vartheta_w - \vartheta_a)$. Assuming that radiative diffusivity can *not* be neglected in the boundary layer, one obtains

$$R = 0.66 \frac{p}{1000} \frac{\vartheta_w - \vartheta_z - \gamma h}{e_w - e_z} \frac{\ln \dfrac{z + z_0}{d + z_0} + \dfrac{k_0 w^* d}{D_1}}{\ln \dfrac{z + z_0}{d + z_0} + \dfrac{k_0 w^* d}{D_2 + K_R}} \qquad (13),$$

where d is the thickness of the boundary layer. The above treatment assumes a discontinuity in the value of the diffusivity at the top of the boundary layer and in the first approximation this assumption appears satisfactory.

With $z = 600$ cm, $z_0 = 0.6$ cm, $d = 0.25$ cm, $w^* = 16$ cm/sec (corresponding to a wind velocity of about 6 m/sec at a height of 6 m), $k_0 = 0.4$, $D_1 = 0.206$ cm^2/sec, $D_2 = 0.181$ cm^2/sec, and $K_R = D_2$, one obtains a ratio, Q_h/Q_e, which is 1.30 times greater than computed by means of equation (11). Thus, Bowen's formula may give too small values of R when applied to atmospheric conditions, but this conclusion is based on inadequate knowledge as to the radiative diffusivity directly above the water surface. A few observations suggest, however, that radiative diffusivity must be considered.[12]

The application of Bowen's formula to atmospheric conditions will be further invalidated if the evaporation from a water surface is greatly increased at wind velocities high enough to carry spray to the air. Montgomery[7] has suggested that this process is of great importance and that as a result of evaporation from spray the evaporation will not remain proportional to the wind velocity, as is nearly the case when transfer by eddy conductivity only is considered, but will increase more rapidly at higher wind velocities. In a formal manner one can state that due to the effect of spray the evaporation will not be proportional to the wind velocity, w, but to w^n, where n is greater than 1.0. Heat conduction, on the other hand, will not be altered by the presence of spray and will remain proportional to the wind velocity. Therefore, the Bowen ratio must be multiplied by a factor, w^{1-n}; that is, at higher wind velocities the Bowen ratio, R_B, gives too high values of R.

The above analysis leads to the conclusion that the Bowen ratio, R_B, when applied to atmospheric conditions, gives only an approximately correct value of the ratio, $R = Q_h/Q_e$. The closeness of the approximation depends upon the importance of radiative diffusivity, the character of the turbulence near the ground, and the effect of spray upon evaporation. At present, none of the processes involved is well enough understood to make possible exact determinations of the ratio R under varying conditions. In the computation of evaporation, the term RL (equation 1) has the character of a correction term, and uncertainty as to the exact value of R does not, therefore, seriously impair the usefulness of the energy equation for determination of evaporation.

DISCUSSION OF THE PAPER

Prof. A. F. Spilhaus (*New York University, New York, N. Y.*):

Meteorologists do not use the Bowen ratio at all, but it is up to them to convince hydrologists that it is unsound and to point out better methods.

Prof. B. Haurwitz (*Massachusetts Institute of Technology, Cambridge, Mass.*):

The value of K_R for the radiative transfer of heat is certainly much larger than 10^3 c.g.s. units according to the new values for the absorption coefficients of water vapor. The above value was computed by Brunt under the assumption that a layer containing 0.3 mm. of precipitable water absorbs completely the so-called W-radiation. Such a layer would have a thickness of 40 meters next to the ground. But according to Elsasser, with the new absorption coefficients the amount of precipitable water should be very considerably larger, which would increase K_R at least by a factor of 10. Moreover, the theory is based on the assumption that the layers of complete absorption of W-radiation are thin. This assumption may be considered correct as long as a layer of 40 meters (0.3 mm. of precipitable water) absorbs completely. But if the layer is ten times thicker, as it should be, at least, in view of the newer absorption coefficients, the whole analogy between radiative and turbulent transfer of heat breaks down. The thickness of the layers considered necessary by Elsasser are too thick for use of K_R in a shallow layer of the ocean.

Dr. H. Wexler (*U. S. Weather Bureau, Washington, D. C.*):

I agree with Professor Haurwitz that the method of using K_R for layers of ocean is inapplicable. It is suggested instead that Elsasser's Radiation Chart (2d ed.) be used to find the radiative heating. Use of Brunts' formula is extremely uncertain in view of the thin layer involved and highly selective character of radiation.

Mr. W. C. Jacobs (*U. S. Weather Bureau, Washington, D. C.*):

The use of K_R affects my own computations seriously only in the expressions for Q_a and Q_c, but not the evaporation.

Prof. N. W. Cummings (*San Bernadino Valley Junior College, Calif.*) communicated a letter to the conference, stating that although he and Dr. Sverdrup were in agreement on most points concerning the Bowen ratio, he would like to outline some points of difference in their views, as follows (read by Professor Spilhaus):

Dr. Sverdrup lays great stress on the transfer of heat by radiation, and urges further investigation of the question. In this, he is, of course, correct. Nevertheless, we must avoid any exaggerated idea of the probable magnitude of the source of error he has in mind. Moreover, we must recognize the intimate relation between radiative diffusivity and the long-wave radiation which is subtracted from the sun and sky radiation in order to get Dr. Sverdrup's Q_r. In the investigations made by the present writer, radiation has been dealt with in a manner which, in the light of Dr. Sverdrup's discussion, may be described as follows:

His quantity K_R has not been entirely neglected, but has been treated as if it were a part of the outgoing long-wave radiation just mentioned. This treatment may not be strictly correct, but it is certainly not as bad as a total neglect of this energy component. The results of the experiments of Cummings and Richardson strongly indicate that Dr. Sverdrup's suggestion of a manyfold error is excessive. In fact, the error seems imperceptible. It should be noted that the experiments were undertaken for the specific purpose of providing an over-all test of the general procedure which involves an application of the Bowen equation.

It is difficult to understand the remarks about spray, contained in the latter part of the paper. There is no obvious reason for supposing that spray constitutes an exception to the generalization pointed out by Bowen, that the transport of mass and of sensible heat follow the same laws. In fact, it seems highly probable that the case in which spray occurs can be analyzed by a method similar to Bowen's case (III).

REFERENCES

1. **Ångström, Anders**
   1920. Applications of heat radiation measurements to the problems of the evaporation from lakes and the heat of convection at their surfaces. Geog. Ann. Stockholm, Heft 3. 16 p.

2. **Bowen, I. S.**
   1926. The ratio of heat losses by conduction and by evaporation from any water surface. Phys. Rev. **27**: 779–787.

3. **Brunt, David**
   1934. Physical and dynamical meteorology. New York and London. 411 p.

4. **Cummings, N. W.**
   1936. Evaporation from water surfaces: status of present knowledge and need for further investigations. Trans. Am. Geophys. Un. **1936**: 507–509.

5. 1940. The evaporation-energy equations and their practical applications. Trans. Am. Geophys. Un. **1940**: 512–522.

6. **Cummings, N. W., & Richardson, Burt**
   1927. Evaporation from lakes. Phys. Rev. **30**: 527–534.

7. **Montgomery, R. B.**
   1940. Observations of vertical humidity distribution above the ocean surface and their relation to evaporation. Papers in Phys. Oceanogr. and Meteor. **7** (4). 30 p.

8. **Mosby, Hakon**
1936. Verdunstung und Strahlung auf dem Meere. Ann. d. Hydrogr. u. Mar. Meteor. **64:** 281–286.

9. **Richardson, Burt**
1931. Evaporation as a function of insolation. Trans. Am. Soc. Civ. Engin. **95:** 996–1011, discussion 1012–1019.

10. **Rossby, C.-G.**
1936. On the frictional force between air and water and on the occurrence of a laminar boundary layer next to the surface of the sea. Papers in Phys. Oceanogr. and Meteor. **4** (3). 20 p.

11. **Schmidt, Wilhelm**
1915. Strahlung und Verdunstung an freien Wasserflächen; ein Beitrag zum Wärmehaushalt des Weltmeers und zum Wasserhaushalt der Erde. Ann. d. Hydrogr. u. Mar. Meteor. **43:** 111–124.

12. **Sverdrup, H. U.**
1936. The eddy conductivity of the air over a smooth snow field. Geofysiske Publikasjoner. **11** (7). 69 p.

13. 1937. On the evaporation from the oceans. Jour. Marine Research **1:** 3–14.

Contribution No. 203

A NOTE ON SOME LABORATORY MEASUREMENTS OF THE TRANSPARENCY OF SEA WATER

BY

R. T. YOUNG JR:

Worcester Polytechnic Institute and Scripps Institution of Oceanography[1]

Laboratory measurements of the absorption of light by sea water have been reported for surface samples from a number of stations in the Atlantic (1, 2, 3, 4). The work of Clarke and James (4), which includes results on 12 stations, both in and off shore for wave-lengths 3650–8000 Å, is of particular interest. They find that for off-shore waters the difference between the absorption of sea water and pure water at all wave-lengths is due practically entirely to suspended materials, termed "suspensoids," which can be filtered out. For off-shore waters these suspensoids are non-selective with respect to wave-length. For in-shore waters two agents are responsible for absorption: suspensoids, responsible for the major part of the absorption, and a filter passing material. In this case both are selective in their action on light. The effect of suspended materials in lake-waters had been previously investigated by Pietenpol (5) and by James and Birge (6). Suspensoids (the term was coined by James and Birge) were found to be the major factor involved in most of the waters they studied. Filter passing material was also present which was highly selective in its absorption of light. It is the purpose of this note to describe some similar measurements on samples obtained down to 60 meters depth in the Pacific Ocean.

The essential features of the absorption apparatus are shown in Figure 32. A parallel beam of light (angular divergence 6′) *L*, 1.2

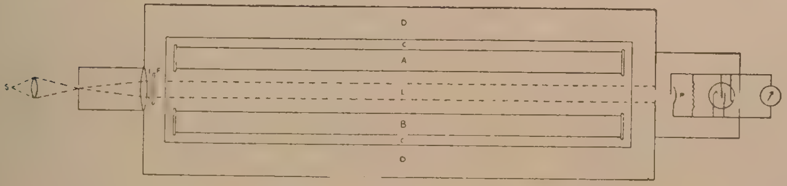


Figure 32. Absorption Apparatus

---

[1] Contributions from the Scripps Institution of Oceanography, New Series No. 203.

Reprint from SEARS FOUND. JOURN. MAR. RES.

cm in diameter, passed through a glass tube 2.5 cm in diameter and 1 meter long. The beam then passed through an aperture 0.8 cm in diameter and was incident on an RCA 917 vacuum photocell, *P*. Two identical tubes, *A* and *B*, were mounted rigidly in the parallel grooves of an optical bench which was fastened to a flat steel plate, *C*. This latter could be moved by means of a rack and pinion parallel to itself across a 10″ channel beam, *D*, so as to bring either of the tubes into the light beam. The ends of the tubes were ground perpendicular to the axes of the tubes to within 30″ and glass flats were pressed against the ends by external collars. The glass to glass contacts between the flats and the tubes were sealed with stop-cock grease. Samples of water in the tubes thus came in contact only with glass. The tubes were filled and emptied through side arms. After the tubes were in adjustment they produced no visible displacement of the light-beam, and for identical samples of water the recorded intensity of light for either tube was the same. The light source was a tungsten filament, 108 watt, 6 volt projection lamp. The photocell current was amplified by a single stage amplifier using an RCA 954 pentode acorn tube in a circuit described by Gabus and Pool (7). The steady plate current was balanced out and the full range of the galvanometer scale utilized for differences in photocell current between the two tubes. With the light intensity used the overall sensitivity was such that a one percent change in intensity corresponded to a 1 cm deflection of the galvanometer. The circuit was capable of much greater sensitivity but stability becomes an increasing problem and for the purposes of this investigation the above sensitivity is sufficient.

A Corning "Sextant Green" filter, *F*, with maximum transmission at 5400 Å was used throughout the course of the experiment, and all the data refer to light of spectral distribution produced by this filter. One tube was filled with twice distilled water from an all glass still. During a period of two months no change in absorption was detected in this sample, showing there was no contamination from the tube. The average of a large number of readings, corrected for reflection losses, gave 84.3% for the transmission of one meter of pure water relative to air. This value is in agreement with the results of James and Birge (6) if one combines their transmission values at various wave-lengths with the transmission values for the filter. (At 5400 Å James and Birge find a transmission of 97%; however the filter passes sufficient light of longer wave-lengths, which are much more strongly absorbed, to reduce this value to that stated above.)

All measurements on sea-water consisted of a comparison of the transmission of a given sample with that of pure water by shifting the tubes back and forth, and the data are in terms of percent transmission relative to pure water. Samples were obtained from the following stations:

Station	*Location: off-shore La Jolla Cove*
A	12.7 miles
B	11.4
C	10.2
D	8.9
E	7.6
F	6.4
G	5.0
H	3.8
I	2.5
J	1.3
K	0.1

At stations A and G samples were obtained every 10 meters down to 60 meters. At the other stations only surface samples were obtained. All samples were stored in glass containers and were tested within 24 to 72 hours after collection. Samples from station A at all depths and surface samples from C and H were passed through a Berkefeld filter. All samples were well ahaken before being placed in the absorption tube. Tests on both filtered and unfiltered samples from station A at 10 meters and from station G, surface, every 24 hours for 4 days showed no significant variation with time. It is estimated that the data on relative transmissions are accurate to within 2%.

The results of the absorption measurements are shown by the curves of Figure 33. The curve for station A (unfiltered), with its

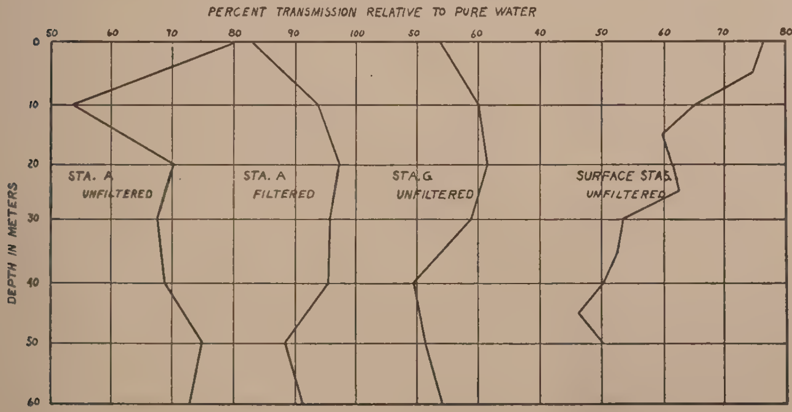


Figure 33. Curves of Relative Transmission.

pronounced minimum is of the same form as many of the curves based on transparency meter data taken in 1938 at many stations in the same general region (8). The existence of a sharp minima in transmissivity seems to be a distinguishing characteristic of the water at many stations. The curve for station G does not correspond to any of the transparency meter curves. At station A the water was shading towards the blue color characteristic of deep ocean waters; at G the water was green, characteristic of inshore waters. The curve for the surface samples, though irregular, shows the water to be increasingly turbid as we come towards shore, which is what we would expect.

The effect of filtering is shown by the filtered curve for station A. With the exception of the surface sample[1] filtering increases the relative transmission of all samples up to 90 to 95% that of pure water, whereas the unfiltered samples ranged from 50 to 80%. Two other samples were also filtered; namely, the surface samples at stations C and H. For these the relative transmission was increased from 65 to 91% and from 53 to 96% respectively. It is of particular interest to note that the sharp maximum of the unfiltered curve has completely disappeared. It is evident that the main factor involved in the absorption of light is material which is stopped by the filter. The porosity of the filter used was such that the majority of particles greater than one micron in diameter were stopped. The residual absorption of the filtered sea water may arise either from suspensoids fine enough to pass through the filter, or from dissolved material.

It may be of interest to report some subsidiary experiments made during the course of this investigation, which give some information concerning the nature of the suspended material. Tests were made on water filtered through a standard mesh used in nets for collecting plankton. These tests showed that any material taken out by the net had little if any effect on the transparency of the water. The relative transmission of the same samples when filtered through the Berkefeld filter was raised from 28 to 88%. Therefore one cannot ascribe the transparency characteristics of sea water to plankton or plankton debris which are removed by a plankton net. This is in line with results obtained with the transparency meter (8), and by others (9, 10), which have shown it is impossible to correlate transparencies with plankton densities.

Another experiment was to allow a column of sea water 35 cm high to stand undisturbed for three days. Measurements were then made on the transmission of the upper and of the lower half. The upper half was only 1% more transparent than the lower. The transmissions of these samples were 68 and 67% that of pure water. Hence there is no appreciable settling of the material responsible for the turbidity of the water. Thus suspensoids can exist in fairly definite layers and produce sharp minima of transparency such as observed at station A.

---

[1] The result for this sample is so much out of line with the rest of the data that it is felt that this particular sample became contaminated in some way.

The author wishes to express his appreciation to Dr. H. U. Sverdrup for the opportunity to carry on this investigation at the Scripps Institution of Oceanography.

REFERENCES

1. Hulburt, E. O.
1926. The Transparency of Ocean water and the Visibility Curve of the Eye. Journ. Opt. Soc. Am. and Rev. Sci. Inst. *13:* 553.
2. Hulburt, E. O.
1928. The Penetration of Ultra-Violet Light into Pure Water and Sea Water. Journ. Opt. Soc. Am. and Rev. Sci. Inst. *17:* 15.
3. Sawyer, W. R.
1931. The Spectral Absorption of Light by Pure Water and Bay of Fundy Water. Conts. Can. Biol. and Fish. n. s. *7:* 75.
4. Clarke, G. L. and James, H. R.
1939. Laboratory Analysis of the Selective Absorption of Light by Sea Water. Journ. Opt. Soc. Am. *29:* 43.
5. Pietenpol, W. B.
1918. Selective Absorption in the Visible Spectrum of Wisconsin Lake Waters. Trans. Wis. Acad. Sci. *19:* 562.
6. James, H. R. and Birge, E. A.
1938. A Laboratory Study of the Absorption of Light by Lake Waters. Trans. Wis. Acad. Sci. *31:* 1.
7. Gabus, G. H. and Pool, M. L.
1937. A Portable Phototube Unit Using an RCA 954 Tube. Rev. Sci. Inst. *8:* 196.
8. Young, Jr., R. T.
1939. Measurements on the Transparency of Sea Water off the Coast of Southern California. J. Mar. Res. *2:* 117.
9. Atkins, W. R. G. and Poole, H. H.
1932. The Photo-electric Measurement of the Penetration of Light of Various Wave-lengths into the Sea and the Physiological Bearing of the Results. Phil. Trans. Roy. Soc. Lond. Ser. B, *222:* 129.
10. Clarke, G. L.
1933. Observations on the Penetration of Daylight into Mid-Atlantic and Coastal Water. Biol. Bull. *65:* 317.

Contribution No. 204

Made in the United States of America

Reprinted from JOURNAL OF BACTERIOLOGY
Vol. 46, No. 1, July, 1943

THE EFFECT OF SOLID SURFACES UPON BACTERIAL ACTIVITY[1]

CLAUDE E. ZOBELL

Scripps Institution of Oceanography, University of California, La Jolla

Received for publication February 15, 1943

The bacterial population of water samples from the sea, lakes or other sources usually increases during storage. While several different factors may be operative (Prescott and Winslow, 1931), it is noteworthy that the magnitude of the increase is often related to the size of the receptacle in which the water is stored. For example, Whipple (1901) found that the bacterial population of water, which initially contained an average of 77 bacteria per ml., increased to 300 per ml. in a gallon, 900 per ml. in a quart, 7,020 per ml. in a pint and 41,400 per ml. in 2-ounce bottles after 24 hours' incubation under comparable conditions. He attributed this to the greater availability of oxygen in the small receptacles which were not filled to capacity. However, ZoBell and Stadler (1940) have shown that the multiplication and respiration of aerobic bacteria is independent of the oxygen tension within the examined ranges of 0.30 to 36 mgm./liter.

Using oxygen consumption as well as bacterial multiplication in glass-stoppered bottles filled to capacity with sea water as criteria, ZoBell and Anderson (1936) noted that bacteria are generally more active in small than in large receptacles of similar shape. Since the small receptacles present relatively more solid surface per unit volume of stored water than large receptacles, they concluded that solid surfaces are beneficial to bacteria in dilute nutrient solutions. A similar conclusion was reached by Lloyd (1937). The following report is concerned with the ways in which solid or adsorbing surfaces may influence bacterial activity.

GLASS SURFACES ADSORB NUTRIENTS

Inasmuch as the effect of volume or solid surfaces upon bacterial activity can be demonstrated only in very dilute nutrient solutions and since the effect is more pronounced with colloidal than with dissolved nutrients, ZoBell (1937) suggested that nutrients may be concentrated on solid surfaces. This explanation is supported by more recent observations here and elsewhere. The work of ZoBell and Grant (1943) shows that bacterial activity is directly proportional to the concentration of nutrients when the latter is less than 10 mgm./l. Since sea water ordinarily contains less than 5 mgm./l. of organic matter (Krogh, 1931), only a small part of which is readily attacked by bacteria (Waksman and Carey, 1935a), it follows that any factor which tends to concentrate the organic matter would promote bacterial activity.

Although the small quantity and complexity of the organic content of sea water make it difficult to estimate the amount which is adsorbed by glass or other solid surfaces under different conditions, there are several ways in which it can be demonstrated that organic material is adsorbed. The sea water used for this purpose was collected in carboys, passed through a fine sintered-glass filter and stored in a water bath at 0°C. The low temperature inhibits (but does not prevent) bacterial activity while the organic matter is being adsorbed.

---

[1] Contribution from the Scripps Institution of Oceanography, New Series No. 204.

The glassware used in the experiments was thoroughly cleaned in hot sulfuric-acid-dichromate-cleaning-solution, treated with trisodium phosphate solution and finally thoroughly rinsed with distilled water. Glass beads and glass wool were heated in a muffle furnace for three hours to free them of organic matter. There is no evidence that the glass carried any nutrients or reactive material. Pyrex glass was used almost exclusively, although no differences were found in the amount of organic matter adsorbed, or in the attachment of bacteria, on Pyrex glass and other kinds of glass.

After standing in sea water at 0°C. for several days, glass slides were removed and stained with methylene blue, gentian violet and carbol fuchsin. Microscopic examination showed the presence of numerous bacteria covering one-fiftieth to one-tenth of the surface. All of the surface was coated with an irregular film of material which stained like complex organic matter. There was little or none of this stained film on slides which were immersed in sea water for only a short period of time. Likewise there was no film on slides immersed in "aged" sea water, the organic content of which had been reduced to less than 0.5 mgm./l.

In other experiments sea water was stored at near 0°C. in 600 ml. bottles partly filled with glass wool, glass beads or with glass tubes to give 2 to 200 cm.^{2} of glass surface per ml. of solution. After different periods of storage the water was carefully siphoned out of the bottles and 100 ml. of dilute permanganate solution was introduced. While heating the bottles in a boiling water bath, they were manipulated to rinse the interior with the oxidizing agent after which they were cooled and the contents titrated with N/80 thiosulfate. The procedure indicated that from 2 to 27 per cent of the organic content of the sea water had been adsorbed by the glass. The amount adsorbed was roughly proportional to the area of solid surface exposed to the water. Repeating this experiment with dilute aqueous solutions or suspensions of known chemical composition showed that glucose, lactate and glycerol were not adsorbed perceptibly, while lignoprotein, nutrose and an emulsified chitin preparation were adsorbed. Very little peptone was adsorbed.

Using similar methods Stark *et al.* (1938) found that measurable amounts of organic matter accumulate within a few hours on chemically clean glass slides immersed in lake water. They expressed the belief that the accumulation of organic nutrients favors bacterial growth. Corroborative evidence is given by the studies of Heukelekian and Heller (1940) on the relation between food concentration and solid surfaces.

Harvey (1941) noted that small glass tubes immersed in sea water adsorbed enough organic matter to alter the surface tension as shown by capillary attraction. Treating a solution containing 10 mgm./l. of peptone with permanganate before and after being exposed to enough glass wool to give a surface of 50 cm.^{2}/

ml. of solution, he found that from 0 to 7 per cent of the peptone was adsorbed by the glass wool. Although the amount of organic matter adsorbed is barely more than the range of the experimental error, Harvey speculates that there might be enough to give a monomolecular layer of nutrient on the glass which might be replaced as fast as it is utilized by bacteria. He points out that according to Blodgett (1935), layers many molecules thick may build up on solid surfaces. A key to the voluminous literature on the factors influencing the adsorption by solids is given by Adam (1938).

BIOLOGICAL EVIDENCE OF ADSORPTION

Immediately after its collection and filtration through fine sintered glass filters, sea water was dispensed in 145 ml. glass-stoppered bottles. Part of the bottles were loaded with several pieces of thin-walled glass tubing tightly packed in an upright position, which materially increased the area of solid surface exposed to water without appreciably decreasing the volume. The oxygen content of the water in some of the bottles was determined at once by a refined Winkler method which was accurate to ±0.01 mgm./l. Part of the bottles were stored in a water bath at 22°C. and part of them were stored in a water bath at near 0°C. Bottles were removed for analysis after 5, 10 and 20 days' incubation. The protocol of a representative experiment is summarized in table 1.

TABLE 1

Oxygen consumed by bacteria after different periods of time in sea water stored at near 0°C. and at 22°C. in glass-stoppered bottles, some of which were filled with glass tubes to increase the area of solid surface

	GLASS-STOPPERED BOTTLES		BOTTLES WITH GLASS TUBES	
	Ratio of cm.^{2}:ml.			
	1.1:1		7.2:1	
	Incubation temperature (°C.)			
	0	22	0	22
Oxygen consumed in 5 days (mgm./l.)	0.02	0.37	0.01	0.46
Oxygen consumed in 10 days (mgm./l.)	0.12	0.78	0.20	0.91
Oxygen consumed in 20 days (mgm./l.)	0.26	1.02	0.38	1.29

As might be expected, bacteria multiplied more rapidly and consumed more oxygen in the water incubated at the higher temperature. At both temperatures more oxygen was consumed in the water which was exposed to the largest solid surface. Previous work has shown that the amount of oxygen consumed is directly proportional to the amount of organic matter oxidized. It requires approximately 1 mgm. of oxygen to oxidize 1 mgm. of organic matter found in sea water. For each milligram of organic carbon which is oxidized to carbon dioxide, 0.45 to 0.65 mgm. of organic carbon is converted into bacterial protoplasm (Waksman and Carey, 1935b, ZoBell and Grant, 1943).

The plate count of the water stored at 0°C. increased progressively throughout the experiment. The plate count of the water stored at 22°C. reached a maximum of several million bacteria per ml. in 5 days and then dropped off sharply after 10 days. Although more oxygen was consumed at 22°C. in the bottles which presented the larger surface area from the 5th to the 20th day, the plate count of this water was smaller than in the bottles having no glass tubes to increase the solid surface. This apparent parodox is explained by the abundance of periphytic or sessile bacteria found attached to the glass. From the direct microscopic examination of the water itself and glass slides immersed in the water, it was calculated that there were more bacteria tenaciously attached to the walls of small glass bottles than the number found in the water.

In earlier experiments ZoBell and Anderson (1936) found 31,000 bacteria per ml. of water and an equivalent of 84,000 bacteria per ml. attached to the glass. Sometimes ten times as many bacteria are found attached to the glass as in the water. The ratio of the number of bacteria occurring in the water and on solid surfaces seemed to be influenced by the concentration and kind of organic matter present, the proximity of the solid surface to the water mass, the time and temperature of incubation and the kinds of bacteria present.

TABLE 2

Oxygen consumed by bacteria in 20 days at 22°C. in sea water, which had been stored in 145 ml. glass-stoppered bottles for 5 days at 0°C.

Part of the water was left in the original bottles in which it was stored and part was transferred to clean bottles and the emptied "original" bottles filled with aged sea water. Aged sea water was incubated in clean bottles as a control.

	INITIAL DISSOLVED OXYGEN CONTENT	DISSOLVED OXYGEN AFTER 20 DAYS	OXYGEN CONSUMED IN 20 DAYS
	mgm./l.	*mgm./l.*	*mgm./l.*
Original water in original bottles	7.83	6.93	0.90
Original water in clean bottles	7.86	7.18	0.68
Aged water in original bottles	7.62	7.23	0.39
Aged water in clean bottles	7.61	7.47	0.14

The water in some of the bottles was carefully siphoned into sterile chemically clean bottles after 5 days storage at 0°C. The original bottles were drained free of sea water and refilled with aged sea water which contained less than 0.2 mgm./l. of bacteriologically oxidizable organic matter. The decanted water with which the bottles were filled originally and the aged sea water in the original bottles was then incubated at 22°C. for 20 days. Oxygen determinations revealed that detectable quantities of oxidizable organic matter had been adsorbed by the glass of the original bottles as shown by the data in table 2. The data are the average of quadruplicates.

It will be noted that whereas the original water, which was incubated in the bottles (original) in which it had been stored for 5 days at 0°C., consumed an average of 0.90 mgm./l. of oxygen in 20 days at 22°C., water siphoned from the original bottles into chemically clean bottles consumed only 0.68 mgm./l. of oxygen. The difference is believed to represent the amount of organic matter adsorbed by the glass walls of the original bottle. This interpretation of results is substantiated by the fact that aged sea water consumed 0.25 mgm./l. more oxygen when incubted in the "original" bottles than when incubated in chemically clean bottles.

Figure 1 shows the number of bacteria found by plate counts in sea water stored in glass-stoppered bottles at 12°C. and the number of attached bacteria. The number of attached bacteria per ml. of water was estimated by counting microscopically those attached per unit area of glass slides submerged in the water and multiplying by the total area of glass exposed to the water. After ten days' incubation the water from part of the bottles was siphoned into "new" chemically clean bottles and the emptied bottles were refilled with "new" recently collected sea water. Determining the oxygen content of water after different periods of incubation showed that little more oxygen was consumed in the decanted or original water in "new" bottles, presumably because a large proportion of its bacterial and organic content had been adsorbed by the original bottle in which it was stored. That both organic matter and bacteria were adsorbed is indicated by the fact that the "new" water in the original bottles consumed more oxygen and consumed it more rapidly than the original water in the original bottles.

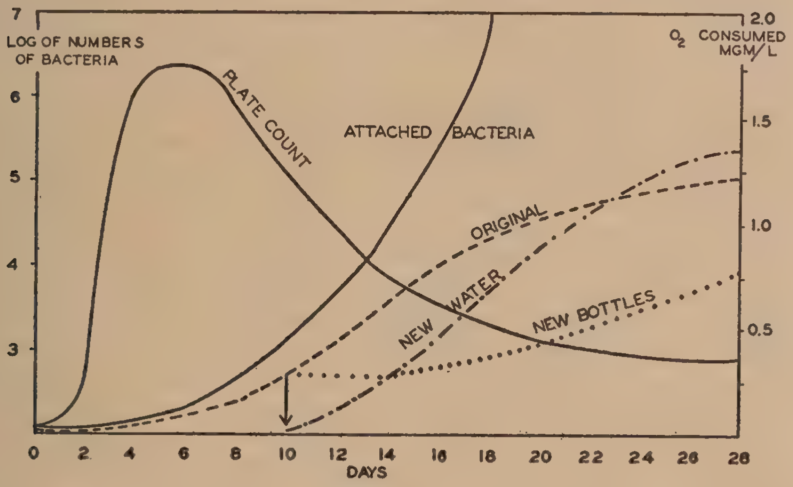


FIG. 1. Number of bacteria found in sea water as indicated by plate counts and the equivalent number per ml. attached to glass surfaces after different periods of incubation at 12°C. (solid lines). The oxygen uptake in the water is also shown (original). After 10 days part of the water was transferred to chemically clean (new) bottles and the emptied bottles were refilled with recently collected (new) water.

The experiment has been repeated several times with similar results. However, in some lots of water the beneficial effect of solid surface upon bacterial activity is barely perceptible, which may be attributable to the quality and quantity of organic matter, the kinds of bacteria present, the surface tension of the water, the nature of the electrolytes and other factors which are being investigated. The explanation of the negative or indifferent results like those obtained by Castell and McDermott (1942), for example, require further experimental work.

The amount of adsorbable organic matter present in natural waters might be influenced by the bacterial population of the water as well as by the quality of the organic matter itself. Part of the organic matter is actually in the form of attached bacterial cells. During the early stages of storage amorphous organic matter appears to predominate on submerged surfaces. After prolonged storage, glass surfaces are usually completely covered with attached bacteria which obliterate any amorphous organic matter which might remain. The work of ZoBell and Grant (1943) indicates that, after prolonged incubation, 60 to 70 per cent of the oxidizable organic carbon in dilute solutions is oxidized to carbon dioxide, and 30 to 40 per cent is converted into bacterial protoplasm.

Our work (ZoBell, *et al.*, 1943) on bacteria which oxidize petroleum hydrocarbons has supplied the most outstanding examples of the beneficial effect of adsorbing surfaces. Due to the low solubility and relative immiscibility in water, hydrocarbons are attacked very slowly by bacteria in aqueous media. If, however, the hydrocarbons are made available on the extensive surfaces of adsorbents such as sand, shredded asbestos or infusorial earth, the rate of bacterial utilization is accelerated two- to ten-fold. Söhngen (1913) noted that ignited soil, silica or iron oxide promoted the bacterial oxidation of petroleum. He concluded that the growth of the bacteria was a function of the solid surface. Similarly Greig-Smith (1914) found that kieselguhr accelerated the utilization of paraffin hydrocarbons by bacteria.

SOME BACTERIA ARE SESSILE

Many species of bacteria grow tenaciously attached to solid surfaces. This is the basis of the buried slide technique of Cholodny (1930) for the direct microscopic study of soil bacteria. It is also the basis of the submerged slide technique of Henrici (1933) for an ecologic survey of water bacteria. The work of ZoBell and Allen (1935), Henrici (1936), Hotchkiss and Waksman (1936), Smith and ZoBell (1937), and Waksman and Vartiovaara (1938) indicates that many water bacteria grow attached to solid surfaces. Kusnetzowa (1937) believes that all water bacteria are capable of attaching themselves to glass. Henrici and Johnson (1935) have described several species of periphytic bacteria which grow only attached to a firm substrate.

Exclusive of exotic species from the terrestrial environment along the littoral zone, most of the bacteria found in the sea appear to be associated with solid surfaces. The microscopic examination of suspended particles reveals the presence of numerous bacteria, whereas very few bacteria are found unattached in sea water. This is in agreement with the observation of Lloyd (1930) that marine bacteria are not planktonic but are attached to solid particles. According to Waksman *et al.* (1933) the bacteria of the sea are usually found attached to larger plankton organisms, existing "only to a very limited extent in the free water of the sea."

The most obvious reason bacteria are found associated with plankton organisms is because the tissue and organic metabolic products of the latter provide a ready source of bacterial food and energy. Also plankton organisms are often coated with a slimy exudate to which bacteria adhere. However, many bacteria do not depend for attachment upon the adhesive properties of solid surfaces, and they are not entirely organotropic because they attach to chemically clean glass, porcelain, plastic, metal and other surfaces. Bacteria will be found tenaciously attached to glass slides an hour or two after submerging the slides in the sea. Such bacteria seem to be stereotropic or thigmotactic.

Upon isolation from submerged slides some of the bacteria grow only on solid surfaces. We have observed sessile bacteria which form a film on the walls of test tubes or flasks without perceptibly clouding dilute nutrient solutions. The most striking example is a chitinoclastic bacterium (ZoBell and Rittenberg, 1938) which grows on strips of chitin with virtually no bacteria occurring in the liquid medium as indicated by the fact that no bacteria may be found in a 2-mm. loopful of fluid although masses of them can be observed growing on solid surfaces.

Achromobacter marinoglutinosus, *Achromobacter membranoformis* and *Flavobacterium amocontactum* are new species of sessile, film-forming or attachment bacteria described by ZoBell and Allen (1935). Dr. Robert S. Breed directs my attention to the fact that since the first two organisms have lophotrichous or polar flagella, according to the revised key they should be designated *Pseudomonas marinoglutinosus* and *Pseudomonas membranoformis*. Among the new species of marine bacteria recently described by ZoBell and Upham (1943) are several sessile forms including *Pseudomonas stereotropis*, *P. sessilis*, *P. periphyta*, *P. coenobios*, *P. membranula*, *Bacillus epiphyticus*, *Micrococcus sedentarius*, *M. sedimenteus*, *Achromobacter stationis*, *A. aquamarinus*, *Bacterium sociovivum* and *B. immotum*.

A total of 96 different cultures of bacteria isolated from marine materials have been used to inoculate sea water in wide mouth bottles enriched with 100 mgm./l. of peptone. Sterile chemically clean glass slides were inserted vertically. After different periods of incubation at 25°C., a slide from each bottle was removed. Without fixation the slides were washed, stained and dried. Large numbers of bacteria were found on slides from 29 of the cultures, virtually no bacteria were found on slides from 47 cultures and a variable number of bacteria were found on slides from 20 cultures. Although the procedure does not differentiate clearly between sessile bacteria and non-sessile ones, it shows differences in the attachment propensities of certain bacterial species which can be duplicated.

No relationship was found between the gram reaction of the pure cultures and their attachment propensities. This is significant because according to Eisenberg (1918), gram-positive bacteria of high lipoid content are adsorbed more readily than gram-negative ones. Rubentschik *et al.* (1936) observed no sharp dividing line between the adsorptive capacity of gram-negative and gram-positive species, although they did note differences in the sessile habits of various species of bacteria.

The development of micro-colonies on glass slides indicates that some of the sessile bacteria multiply while attached to solid surfaces. In very dilute nutrient solutions more bacteria will often be found growing on the walls of the culture receptacle and on immersed glass slides than in the solution (ZoBell and Anderson, 1936).

HOW BACTERIA ATTACH

Sessile bacteria do not seem to be covered with a mucilaginous substance which causes them to stick to solid surfaces with which they come into contact. This point has been investigated by immersing glass slides in suspensions of bacteria and removing them after different periods of time for microscopic examination. Very few bacteria attach to slides firmly enough to resist being washed off with running water unless the slides have been left in contact with the bacteria in dilute nutrient solutions for at least a few hours. Likewise only a small proportion of the bacteria transferred to glass slides and air dried, as in the preparation of a smear for staining, adhere to the glass without heat or chemical fixation. However, when the glass slides are left in dilute nutrient solutions of bacteria for several hours, many bacteria adhere so tenaciously that they are dislodged neither by washing with water nor by staining procedures.

The foregoing observations suggest that bacteria grow on, or attach themselves to, solid surfaces rather than merely being passively stuck. Credibility is lent to this conclusion by the fact that regardless of the density of the bacterial population, more bacteria attach to glass slides during the early logarithmic phase of growth than during later growth phases.

Henrici and Johnson (1935) have described a group of bacteria "which secrete stalks by which they are attached to a firm substrate." Their search of the older bacteriological literature has shown that similar types have been observed before. We have found bacteria growing on slides submerged in the sea and in Great Salt Lake (Smith and ZoBell, 1937) which have a definite holdfast, some of which could be described as having "stalks."

As pointed out by Henrici and Johnson (1935), it is often difficult to distinguish bacteria having large polar or adherent lophotrichous flagella from stalked bacteria on slides which, after several days submergence, are covered with various kinds of bacteria besides other sessile organisms and a heterogeneous assortment of particulate and amorphous organic matter. Also in some cases we have not been certain that some of the definitely stalked microorganisms appearing on submerged slides were bacteria although, except for the stalk, they had the morphological appearance of bacteria. However, in spite of the difficulties, careful microscopic study of submerged slides has convinced us that some water bacteria are attached to solid surfaces by means of a filament or stalk which is definitely longer and narrower than the bacterial cell itself. In fact, some stalked bacteria have been observed which appeared to be dividing.

On only three slides out of several hundred examined have we observed bacteria with stalks which were sufficiently rigid to hold the bacteria away from the slide. When examined as wet mounts the bacteria appeared to be a few microns away from the slide. It was necessary to focus up and down in order to follow the stalk from the holdfast to the bacterial cell.

Several cultures of sessile bacteria have been isolated from submerged slides. After a few hours submergence in the sea the glass slides were washed with sterile water, transferred aseptically to sterile petri dishes and covered with nutrient agar (ZoBell, 1941). When grown in sea water enriched with 5 mgm./l. each of glucose and peptone, several of these cultures contained bacteria which attached to glass slides immersed in the medium. Six of them had structures which appeared to be stalks, but with all six cultures the bacterial cell, the stalk and the holdfast rested directly upon the glass slide. Upon cultivation on solid media they lose their stalks, although this property is regained after a few passages through sea water containing less than 10 mgm./l. of organic matter.

Only a small percentage of the cells of the six "stalked" cultures found attached to glass slides exhibited stalks. This may be attributed to faulty technique in staining stalks; it may suggest that the stalks develop after the cells have attached, or it is possible that all the cells do not have stalks. Further studies will be necessary to clarify this point.

Although some bacteria are attached by stalks, this is not the commonest attachment structure. Most sessile bacteria are found with the bacterial cells in intimate contact with the solid surface. It is believed that after coming into contact with a solid surface, physiologically active sessile bacteria secrete a cementing substance. When the bacteria are removed mechanically from glass slides to which they have attached themselves, a faintly staining film having the shape and arrangement pattern of the attached cells frequently remains on the slides.

Some bacteria appear to secrete a faintly staining material on the slide which is two or three times the diameter of the cells themselves. Examining slides after different periods of submergence in bacterial cultures reveals that the size of this film increases with age. It is not uncommon to see micro-colonies on "islands" of the film. The smaller islands conform more or less to the shape of the cells but larger islands of the film are quite irregular in outline.

The film appears to be a part of or product of the bacterial cell. The fact that the islands are most pronounced in dilute nutrient solutions makes it seem probable that the film does not consist of organic matter adsorbed from the water. However, it is still indeterminate whether the bacteria are responsible for the presence of the film or if the film of adsorbed organic matter precedes the bacteria thereby providing for their attachment. Microscopic particles of detritus including particles of carbon and indigotin, with which the slides have been treated, stick to the film thereby establishing that the film has adhesive properties. This mucilaginous slime produced by sessile bacteria is believed to be instrumental in the fouling of submerged surfaces (ZoBell and Allen, 1935, ZoBell, 1939). According to Sanborn (1932), the formation of slimy or viscous growths is common in cultures from marine fish.

An effort has been made to characterize the holdfasts of sessile bacteria by treating slides coated with attached bacteria with different solvents. Dilute HCl or H_2SO_4 has little or no effect on the holdfasts, which indicates that the cementing or adhesive material is not calcareous. (Many sessile organisms attach by means of calcareous cements which are dissolved by dilute acids.) Dilute caustic, ammoniacal or bicarbonate solutions were without apparent effect. The holdfast is not destroyed by 95 per cent alcohol. Xylol, chloroform, ether and carbon disulfide seemed to dislodge some of the attached bacteria but not enough to warrant the conclusion that the cement is a lipin. Eisenberg (1918) believed that a high lipin content favored the adsorption of bacteria.

None of the common staining solutions dislodged the attached bacteria. When dessicated, many of the attached bacteria disappear from slides. Recently it has been found that a bacterial chitinase extract dislodges many attached bacteria which suggests that the holdfast is chitinous, but the extract also contains proteolytic and possibly other exoenzymes which themselves might have digested the cementing material.

Dilute solutions of detergents or wetting agents such as sodium taurocholate, sodium ricinoleate, sodium lauryl sulfate (Dreft) or "aerosol" remove part of the attached bacteria, the cells of some cultures being affected more than those of others. The presence of detergents or surface tension depressants in dilute nutrient solutions retards the attachment of bacteria to solid surfaces.

When submerged in the sea the number of bacteria found on glass slides increases exponentially with time until the bacteria became too numerous to count or are obscured by other organisms and detritus which attach simultaneously. This suggests that the bacteria are multiplying on the glass or that the attachment of bacteria promotes the attachment of additional bacteria. Probably the adhesive films formed by the first sessile bacteria help other bacteria which come into contact with the surface to gain lodgement long enough to produce their own holdfasts. If only the cumulation of adsorbed nutrients was responsible, the increase in the number of bacteria on submerged slides would probably be arithmetic rather than exponential.

EFFECT OF ORGANIC NUTRIENTS

Unquestionably organic nutrients on the solid surfaces promote the multiplication of bacteria but there is evidence that adsorbed nutrients are not directly responsible for the attachment of bacteria. Slides have been left in sterile sea water until they have been covered with adsorbed organic matter after which they were submerged in the sea. Significantly more bacteria did not attach to these slides than to chemically clean ones.

However, the organic content of the water has a pronounced effect on the relationship of microorganisms to solid surfaces. Increasing the solid surface area ordinarily does not alter the rate of bacterial multiplication or metabolism in solutions containing more than 5 to 10 mgm./l. of organic nutrients. The beneficial effect of solid surface becomes more pronounced as the concentration of organic nutrients decreases. Heukelekian and Heller (1940) observed that *Escherichia coli* failed to grow when the concentration of organic nutrients was less than 0.5 to 2.5 mgm./l. except in the proximity of solid surfaces. When the concentration exceeded 25 mgm./l., the activity of *E. coli* was not influenced perceptibly by solid surfaces.

The kind of organic matter is also important. Increasing the ratio of solid surface to volume does not influence the rate of bacterial activity in dilute solutions (1.0 to 10 mgm./l.) of glucose, glycerol or lactate. Many bacteria are found attached to glass slides in such solutions, but in such solutions the bacteria do not seem to depend upon solid surfaces because proportionately more occur suspended in the solution than anchored. Conversely in solutions enriched with 5 mgm./l. of sodium caseinate, lignoprotein or an emulsified chitin preparation inoculated with a few bacteria per ml., a beneficial effect of increased solid surface can be demonstrated. This suggests that there is a relationship between solubility, dispersion or molecule size of the organic nutrient and the surface phenomenon. However, many other factors may be involved and further studies must precede generalizations.

The beneficial effect of solid surface upon the rate of bacterial activity in sea water is much more pronounced with some lots of sea water than with others. Since the inorganic content, gas tension and the pH of sea water have been nearly constant, the differences are attributed to differences in the organic content of the water. A heterogenous assortment of organic matter occurs in sea water but most of it probably consists of large molecules of colloidal dimensions which are fairly refractory to bacteria decomposition. Differences may be expected in the relative quantities of different kinds of organic matter adsorbed on glass, and especially differences in the orientation of molecules. Their availability might be influenced by the angle of adsorption as well as by the polarity, both of which might influence the vulnerability of hydroxy, carboxy, amino or other groups. According to Adam (1938) most organic molecules form zero angles with glass but certain large molecules are "adsorbed on to glass with the long chains outwards." For example, the contact angle of paraffin wax molecules is 105°, the largest found with solids and water.

It is generally believed that the large molecules or particulate materials are hydrolyzed by exoenzymes before the organic matter can be ingested and assimilated by bacteria. In a dilute solution of such organic hydrolytes, containing only a relatively few bacteria, the bacteria would have to be in close contact with the hydrolyte in order for the exoenzymes to be most effective. Otherwise the bacterial exoenzymes would be dissipated throughout the solution. It is conceivable that the hydrolyzates themselves might have a tendency to diffuse away from the bacterial cell responsible for their formation; so, in dilute nutrient solutions of large molecules of organic matter, the bacteria may have difficulty in digesting and absorbing or ingesting enough nutrient to provide for their organic requirements.

However, if the bacterial cell is on a solid surface juxtaposed with nutrient hydrolytes, the solid surface may retard the diffusion of the exoenzymes and the hydrolyzates away from the cell (see figure 2). Besides the physical attraction which might be exerted by the solid surface, an anchored bacterium would not be influenced by the bombardment of molecules (with the resultant Brownian movement and diffusion) which would tend to separate the bacterium from its exoenzymes and hydrolyzates. Furthermore, the interstices at the tangent of the bacterial cell and solid surface might serve as concentration foci for exoenzymes and hydrolyzates. As other bacteria attach or develop from cell division, more interstitial or capillary spaces would be formed which would tend to retard further the diffusion of materials away from the cells and to favor the absorption or ingestion of soluble nutrients. Also solid surfaces might facilitate the orientation of bacterial exoenzymes in the most advantageous position (Danielli, 1937). According to Adam (1938), it has been found by Willstätter that adsorption of a soluble enzyme on a neutral inactive support will often increase its stability and activity.

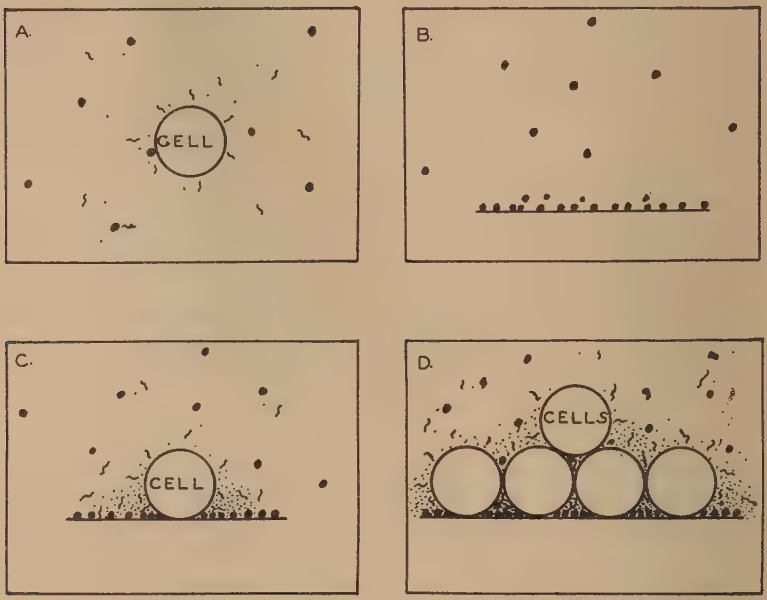


FIG. 2. A, A free-floating bacterial cell surrounded by a few suspended particles of food (dark circles) which must be hydrolyzed by the exoenzyme (helicoidal lines) before the resulting hydrolyzates (dots) can be ingested and assimilated. B, Particles of food concentrated in a monomolecular layer on a solid surface. C, Food particles are more available to the cell on the solid surface where the interstices at the tangent of the bacterial cell and the solid surface retard the diffusion of exoenzymes and hydrolyzates away from the cell. D, Multiple cells form additional interstitial spaces.

From their studies on the relation of solid surfaces to bacterial activity, Heukelekian and Heller (1940) conclude that "Surfaces enable bacteria to develop in substrates otherwise too dilute for growth. Development takes place either as a bacterial slime or colonial growth attached to the surfaces. Once a biologically active slime is established on surfaces, the rate of biological reaction is greatly accelerated."

KINDS OF SOLID SURFACES

In order to appraise the effect of surface upon bacterial activity the solids used must be chemically inert. Glass has proved best for this purpose because, besides being virtually insoluble, it is available in a great variety of forms and shapes, and transparent glass slides lend themselves readily to the direct microscopic studies of attached materials. However, glass is by no means the only substance which can be used to demonstrate the attachment of bacteria and the growth-promoting properties of solid surfaces.

When submerged in the sea, bacteria attach to celluloid, cellophane, vinylite, lucite, bakelite and other plastics both transparent and opaque. There is a marked difference in the rate of attachment of bacteria to, the accumulation of organic matter on, and the growth-promoting properties of different kinds of plastics. Details can be given only after war-time restrictions are lifted because these studies may have direct bearing upon the "fouling" of ships' bottoms and other submerged surfaces (ZoBell, 1939). There is evidence that some plastics are attacked biochemically by bacteria.

Bacteria attach less readily to solid surfaces which are rendered hydrophobic by the application of greases, waxes and certain kinds of lacquers than to hydrophilic surfaces of low surface tension which are more wettable. Likewise positively charged surfaces are more conducive to the attachment of bacteria than negatively charged ones. Neither color, plane or angle of immersion nor the degree of roughness or smoothness of a surface seems to influence the attachment of bacteria. For unexplained reasons bacteria generally attach more readily near the edge of glass slides than in the center regardless of the position in which the slides are immersed. Bacteria attach to gold and silver surfaces but clean silver, copper, aluminum and iron surfaces appear to exert an adverse oligodynamic effect.

Porcelain surfaces also promote bacterial activity in dilute nutrient solutions, unglazed porcelain particularly. Ignited sand, asbestos fibres, emery grit and kieselguhr are likewise beneficial. It has been demonstrated that these substances adsorb organic matter from sea water, and proportionately many more bacteria are associated with the solid particles than are found in similar volumes of water. The bacteria are usually so tenaciously attached to the solid particles that oxygen consumption or similar tests must be used to demonstrate their presence and activity. Indifferent results have been obtained with bentonite, talc, kaolin and various samples of marine clays although they adsorb organic matter. This may be because particles smaller than bacteria adsorbed on bacteria may be inimical as suggested by the work of Peele (1936).

Conn and Conn (1940) found that bentonite, kaolinite, beidelite and illite stimulated bacterial activity, which they attributed to increased surface and other factors. Bigger and Nelson (1941) found that ignited asbestos, barium sulfide, barium hyposulfite, barium sulfite, tricalcium phosphate, ferric phosphate, ferrous silicate, kaolin, kieselguhr, magnesium silicate, manganese dioxide, permutit, silica, silver sand, soil, unglazed porcelain, talc and zirconium silicate make it possible for coliform bacteria to grow in very dilute nutrient solutions (less than 0.4 mgm./l. of organic nutrient). Although some of the substances used by Bigger and Nelson may be active in other ways, they attribute the beneficial effect to the concentration of nutrients by the substances.

Studies with sand, kieselguhr and similar substances reveal that another factor is involved, namely, the distribution of the solid surface in the solution. For example, a layer of sand or kieselguhr 2 to 3 cm. thick in the bottom of a bottle accelerates bacterial activity little more than does a layer only 2 to 3 mm. thick, although the thicker layer presents ten times as much surface area, presumably because little more of the solution is in intimate contact with the sand in one case than in the other. In order to be most effective the solid surface must be distributed throughout the solution. This has been demonstrated by using different shapes of glass.

Enough 2 mm. glass beads were placed on the bottom of 145 ml. glass-stoppered bottles to provide the same surface area as provided by a dozen pieces of 6 mm. glass tubes standing upright in similar bottles. In the latter arrangement none of the water was more than 3 mm. from a solid surface whereas in bottles partly filled with glass beads much of the water was considerably more than 3 mm. from a solid surface. Bacteria consumed oxygen more rapidly in the bottles with the glass tubes than in those having a similar area of glass beads. In fact, the glass tubes stimulated bacterial activity more than a 2- to 3-cm. layer of silica sand in the bottom of the bottles. Although the latter presented much more solid surface than the glass tubes, it was not in close proximity to the water.

Glass wool has been used to provide large areas of solid surface throughout the bottles of water, but it has not proved to be entirely satisfactory for experimental purposes because it is difficult to wash free of impurities, and it is virtually impossible to free the glass wool of adherent gas bubbles.

According to Prescott and Winslow (1931), Kohn conducted experiments which suggested that the multiplication of bacteria during storage in glass receptacles is attributable partly to the solution of certain constituents from glass which favor bacterial life. While this may be true of certain waters, there is no evidence that the waters which we have examined dissolve any growth-promoting substance. The beneficial effects of surface observed with Pyrex glass have been comparable to those obtained with various kinds of soft glass. When stored at 0°C. in contact with large surface areas of different kinds of glass including glass wool and glass beads, the water is rendered less growth-promoting rather than more so which, as discussed above, is due to the adsorption of organic nutrients. That nothing toxic is dissolved from the glass is shown by the fact that such water regains its growth-promoting properties upon the addition of a fraction of a mgm./l. of glucose. It is not a reflection upon the work of Kohn to suppose that the growth-promoting constituents dissolved from glass were organic compounds because, as emphasized by Adam (1938), it is very difficult to prepare chemically clean glass surfaces. Soft glass is more difficult to clean than harder glasses, and significantly, Kohn noted the greatest bacterial increases in bottles made of softer glasses. As noted by Esty and Cathcart (1921) unbuffered media have a tendency to become more acid during heat sterilization in Pyrex glass and more alkaline in soft glass tubes, but in all of our observations on the effect of glass surfaces we have used well buffered media.

DISCUSSION

Conn and Conn (1940) speculate that besides concentrating nutrients, solid surfaces may adsorb toxic products and increase the availability of oxygen. These two factors may be operative under certain conditions but it is doubtful if either factor helps to account for the beneficial effect of solid surfaces in extremely dilute solutions.

For many years more or less inert solids such as chalk, glass beads, glass wool, sand, kaolin, clinkers, sponge, animal tissue, charcoal, iron nails, cellulose, etc., have been employed in liquid media to promote the growth of bacteria (see literature cited by Breden and Buswell, 1933). Some of the substances probably provide a favorable oxygen tension and others may serve as a support for bacteria. Breden and Buswell (1933) point out that in continuous fermentation processes (vinegar, sewage beds, etc.), inert materials support organisms, "preventing their loss with removal of spent liquor and making possible the heavy inoculation of fresh substrates." They found that shredded asbestos improved methane fermentation. Numerous methane-producing organisms grew on the asbestos, small pieces of which could be used to inoculate successfully new batches of soluble substances. Butterfield (1937) attributed the beneficial effect of activated sludge to the adsorption of dissolved and colloidal materials.

Peele (1936) investigated several factors, including electric charges and base-exchange complex, which influence the adsorption of bacteria by soils. He found that the addition of soil to bacterial suspensions sometimes retarded the evolution of carbon dioxide, which he attributed to the adsorption of bacteria by soils. Similar results were obtained by Chudiakow (1926). Clay particles considerably smaller than bacteria predominated in the soils used by Peele and Chudiakow, so it is conceivable that such particles surrounding the bacteria retarded the assimilation of nutrients or other normal bacterial activities. McCalla's (1940) observation on the large number of active adsorption sites on a bacterial cell, which may have an affinity for minute positively-charged particles, supports such a concept. According to Dianowa and Woroschilowa (cited by Peele, 1936), "the decrease in biochemical activity when a portion of soil was shaken with a culture of bacteria was much greater when the soil was made up of very fine particles, such as silt and clay, than when the soil contained a larger proportion of sand." Also the adsorption of bacteria by soil probably caused them to settle to the bottom of the medium where they would be less active than when distributed throughout the medium. Peele used *Azotobacter chroococcum* as the test organism.

On the other hand Söhngen (1913) records that the activities of hydrocarbon-oxidizing bacteria are accelerated by adsorption by soil. According to Rubentschik *et al.* (1936) the activity of some bacteria (nitrifiers) is retarded while that of others (sulfate reducers) is increased by adsorption by lake mud.

In their studies on the adsorption of bacteria by inert reagents, Gunnison and Marshall (1937) found no evidence that the beneficial clinical effects which sometimes follow the oral administration of kaolin, charcoal, Fuller's earth, etc., could be attributed to the inactivation of adsorbed pathogens. They believe that the adsorption of toxins or enzymes is more likely to account for the reported clinical improvements than the removal of bacteria by adsorption. In their experiments, like those of Peele (1936) and Chudiakow (1926) with soils, the particles used were mostly smaller than bacteria and the concentration of nutrients was high. In our experiments a beneficial effect of solid surface has been observed only in very dilute nutrient solutions and on solid surfaces which are large in comparison to the size of bacteria.

Further studies on the influence of particle size, food concentration and composition, the behavior of different kinds of bacteria, electric charge, surface tension, electrolytes, pH and other factors which influence the relation of bacteria to solid surfaces are indicated.

SUMMARY

Minute but demonstrable quantities of organic nutrients are adsorbed from sea water by glass.

In dilute nutrient solutions such as sea water the organic matter concentrated by adsorption on solid surfaces enhances bacterial activity.

Many of the bacteria found in sea water are sessile, growing exclusively or preferentially attached to a solid surface.

Most sessile bacteria appear to grow on solid surfaces by exuding a mucilaginous holdfast. A few have stalks. The exudate may aid in concentrating nutrients in the vicinity of the attached bacteria.

The beneficial effect of solid surfaces is usually evident only in very dilute nutrient solutions (less than 10 mgm./l). The beneficial effect is more pronounced with colloidal or poorly soluble substances than with those which are very soluble.

It is believed that besides concentrating nutrients by adsorption and providing a resting place for sessile bacteria, solid surfaces retard the diffusion of exoenzymes and hydrolyzates away from the cell thereby promoting the assimilation of nutrients which must be hydrolyzed extracellularly prior to ingestion.

Glass, plastics, porcelain, sand, kieselguhr and other inert particulate materials are surface active. Particles larger than bacteria are more beneficial than smaller particles. Particles smaller than bacteria may be harmful.

In order to be most effective the solid surface must be distributed throughout the dilute nutrient solution.

ACKNOWLEDGMENTS

It is a pleasure to acknowledge the assistance of Catharine B. Feltham Esther C. Allen, D. Q. Anderson, D. M. Michener and S. C. Rittenberg who aided in the experimental work reported in this paper. Helpful suggestions

have been received from Dr. Blodwen Lloyd, Austin Phelps, Carroll W. Grant and others.

REFERENCES

Adam, N. K. 1938 The Physics and Chemistry of Surfaces. Clarendon Press, Oxford, 402 pp.

Bigger, J. W., and Nelson, J. H. 1941 The growth of coliform bacilli in distilled water. J. Path. Bact., **53**: 189–206.

Blodgett, K. B. 1935 Films built by depositing successive monomolecular layers on a solid surface. J. Am. Chem. Soc., **57**: 1007–1022.

Breden, C. R., and Buswell, A. M. 1933 The use of shredded asbestos in methane fermentations. J. Bact., **26**: 379–383.

Butterfield, C. T. 1937 The oxidation of sewage by bacteria in pure culture. J. Bact., **34**: 207–219.

Castell, C. H., and McDermott, L. A. 1942 Multiplication of bacteria in water and its significance in food spoilage. Food Research, **7**: 244–253.

Cholodny, N. 1930 Über eine neue Methode zur Untersuchung der Bodenflora. Arch. Mikrobiol., **1**: 620–652.

Chudiakow, N. N. 1926 Über die Adsorption der Bakterien durch den Boden und den Einfluss derselbe auf die mikrobiologischen Bodenprocessen. Zentr. Bakt. Parasitenk., II, **68**: 345–358.

Conn, H. J., and Conn, Jean E. 1940 The stimulating effect of colloids upon the growth of certain bacteria. J. Bact., **39**: 99.

Danielli, J. F. 1937 The relations between surface pH, ion concentrations and interfacial tension. Proc. Roy. Soc. (London), B 122: 155–174.

Eisenberg, P. 1918 Ueber spezifische Adsorption von Bakterien. Zentr. Bakt. Parasitenk., II, **81**: 72–104.

Esty, J. R., and Cathcart, P. H. 1921 The change in the hydrogen-ion concentration in various mediums during heating in soft and pyrex glass tubes. J. Infectious Diseases, **29**: 29–39.

Greig-Smith, R. 1914 The destruction of paraffin by *Bacillus prodigiosus* and soil organisms. Proc. Linnean Soc. N. S. Wales, **39**: 538–541.

Gunnison, J. B., and Marshall, M. S. 1937 Adsorption of bacteria by inert particulate reagents. J. Bact., **33**: 401–410.

Harvey, H. W. 1941 On changes taking place in sea water during storage. J. Marine Biol. Assoc. United Kingdom, **25**: 225–233.

Henrici, A. T. 1933 Studies of freshwater bacteria. I. A direct microscopic technique. J. Bact., **25**: 277–286.

Henrici, A. T., and Johnson, Delia E. 1935 Studies of freshwater bacteria. II. Stalked bacteria, a new order of Schizomycetes. J. Bact., **30**: 61–93.

Henrici, A. T. 1936 Studies of freshwater bacteria. III. Quantitative aspects of the direct microscopic method. J. Bact., **32**: 265–280.

Hotchkiss, Margaret, and Waksman, S. A. 1936 Correlative studies of microscopic and plate methods for evaluating the bacterial population of the sea. J. Bact., **32**: 423–432.

Heukelekian, H., and Heller, A. 1940 Relation between food concentration and surface for bacterial growth. J. Bact., **40**: 547–558.

Krogh, A. 1931 Dissolved substances as food of aquatic organisms. Rapports et Proces-Verbaux des Reunions, **75**: 7–36.

Kusnetzowa, Z. I. 1937 Methode der Ausscheidung der Plankton- und Periphytonbakterien und ihre Anwendung zum Studium der Dynamik der bakteriologischen Prozesse im Wasserbecken. Arbeiten der Limnologischen Stat. Zu Kossino, **21**: 89–103 (Russian with German summary).

Lloyd, Blodwen 1930 Bacteria of the Clyde Sea area: A quantitative investigation. J. Marine Biol. Assoc. United Kingdom, **16**: 879–907.

Lloyd, Blodwen 1937 Bacteria in stored water. J. Roy. Tech. Coll. (Glasgow), **4:** 173–177.

McCalla, T. M. 1940 Cation adsorption by bacteria. J. Bact., **40:** 23–32.

Peele, T. C. 1936 Adsorption of bacteria by soils. Cornell Univ., Agr. Expt. Sta., Memoir **197:** 1–18.

Prescott, S. C., and Winslow, C. E. A. 1931 Elements of Water Bacteriology. John Wiley & Sons, New York, 219 pp.

Roll, H. 1939 Zur Terminologie des Periphytons. Arch. Hydrobiol., **35:** 59–69.

Rubentschik, L., Roisin, M. B., and Bieljansky, F. M. 1936 Adsorption of bacteria in salt lakes. J. Bact., **32:** 11–31.

Sanborn, J. R. 1932 Marine bacteria commonly found on fresh fish. J. Bact., **23:** 349–351.

Smith, W. W., and ZoBell, C. E. 1937 Direct microscopic evidence of an autochthonous bacterial flora in Great Salt Lake. Ecology, **18:** 453–458.

Söhngen, N. L. 1913 Einfluss von Kolloiden auf mikrobiologische Prozesse. Zentr. Bakt. Parasitenk., II, **38:** 621–647.

Stark, W. H., Stadler, J., and McCoy, E. 1938 Some factors affecting the bacterial population of freshwater lakes. J. Bact., **36:** 653–654.

Waksman, S. A., and Carey, C. L. 1935a Decomposition of organic matter in sea water by bacteria. I. Bacterial multiplication in stored sea water. J. Bact., **29:** 531–543.

Waksman, S. A., and Carey, C. L. 1935b Decomposition of organic matter in sea water by bacteria. II. Influence of addition of organic substances upon bacterial activity. J. Bact., **29:** 545–561.

Waksman, S. A., Reuszer, H. W., Carey, C. L., Hotchkiss, M., and Renn, C. E. 1933 Studies on the biology and chemistry of the Gulf of Maine. III. Bacteriological investigations of the sea water and marine bottoms. Biol. Bull., **64:** 183–205.

Waksman, S. A., and Vartiovaara, U. 1938 The adsorption of bacteria by marine bottom. Biol. Bull., **74:** 56–63.

Whipple, G. C. 1901 Changes that take place in the bacterial contents of waters during transportation. Tech. Quart., **14:** 21–29.

ZoBell, C. E. 1936 Periphytic habits of some marine bacteria. Proc. Soc. Exptl. Biol. Med., **35:** 270–273.

ZoBell, C. E. 1937 The influcnce of solid surface upon the physiological activities of bacteria in sea water. J. Bact., **33:** 86.

ZoBell, C. E. 1939 Primary film formation by bacteria and fouling. Collecting Net, **14:** 105–106.

ZoBell, C. E. 1939 The role of bacteria in the fouling of submerged surfaces. Biol. Bull., **77:** 302.

ZoBell, C. E. 1941 Studies on marine bacteria. I. The cultural requirements of heterotrophic aerobes. J. Marine Research, **4:** 42–75.

ZoBell, C. E., and Allen, Esther C. 1935 The significance of marine bacteria in the fouling of submerged surfaces. J. Bact., **29:** 239–251.

ZoBell, C. E., and Anderson, D. Q. 1936 Observations on the multiplication of bacteria in different volumes of stored sea water and the influence of oxygen tension and solid surfaces. Biol. Bull., **71:** 324–342.

ZoBell, C. E., and Rittenberg, S. C. 1938 The occurrence and characteristics of chitinoclastic bacteria in the sea. J. Bact., **35:** 275–287.

ZoBell, C. E., and Stadler, Janice 1940 The effect of oxygen tension on the oxygen uptake of lake bacteria. J. Bact., **39:** 307–322.

ZoBell, C. E., and Grant, C. W. 1943 Bacterial utilization of low concentrations of organic matter. J. Bact., **45:** 555–564.

ZoBell, C. E., Grant, C. W., and Haas, H. F. 1943 Marine microorganisms which oxidize petroleum hydrocarbons. Bull. Am. Assoc. Petroleum Geol., In press.

ZoBell, C. E., and Upham, H. C. 1943 A list of marine bacteria including descriptions of sixty new species. Bull. Scripps Inst. Oceanogr., tech. ser., **5:** 239–345.

Contribution No. 205

Reprinted from THE JOURNAL OF EXPERIMENTAL ZOOLOGY
Vol. 93, No. 2, July, 1943

BIOLOGY OF THE CALIFORNIA SEA-MUSSEL (MYTILUS CALIFORNIANUS)

II. NUTRITION, METABOLISM, GROWTH AND CALCIUM DEPOSITION [1]

DENIS L. FOX AND WESLEY R. COE
Scripps Institution of Oceanography, University of California, La Jolla

FOUR FIGURES

In a recent paper on the influence of temperature, food supply, sex and age on the rate of growth of this species, it was shown that on the coast of Southern California the mussel under favorable conditions may reach a length of 80 to 86 mm. at the age of 1 year, 120 mm. when 2 years old and approximately 145 mm. at the end of the third year (Coe and Fox, '42). The habitat, food, digestion and salinity requirements have been investigated by Fox et al. ('36); the rate of water propulsion has been studied by Fox, Sverdrup and Cunningham ('37), and the changes in tissue chloride in response to heterosmotic environments by Fox ('41). The method of feeding has been reported by MacGinitie ('41), the metabolism of the carotenoid pigments by Scheer ('40), the seasons of spawning by Whedon ('36) and by Young ('42), and the distribution of the species in relation to the salinity of the environment by Young ('41). Whedon and Sommer ('38) have studied the respiratory exchange at different temperatures. Shelford et al. ('35) stress the importance of turbulence on the distribution of the species.

It now remains to consider some of the substances of which the body and shell of the mussel are composed, the amounts and sources of the materials which have contributed to the growth of the tissues and the deposition of the shell as well as the various ways in which the metabolism of the mussel affects the associated organisms in the vicinity.

---

[1] Contributions from the Scripps Institution of Oceanography, New Series no. 205.

This study has included oberservations and experiments on the rate of increase in weights both of somatic tissues and of the gonads in various states of maturity, also the rate of increases in size and weight of shell with special reference to the amount and source of the calcium accumulated.[2] Further studies have been made on the influence of environmental conditions in nature on the rate of growth and on the shape and thickness of the shell, as well as of the effects of various artificial diets and of starvation under experimental conditions.

With reference to nutrition of the mussel it has been necessary to determine the amounts of water, organic substance and calcium contained in the bodies and shells of individuals at successive ages from 2 months to 3 years. The nutritive materials must obviously be sufficient also for the production of a quantity of reproductive cells amounting to fully four-fifths of the weight of the somatic tissues during the first year and approximately equal to the weight of the somatic tissues during the second and third years. Consequently, the gonads of both sexes have been analyzed relative to their content of water, organic matter, ash and calcium.

Having determined the amounts of the various constituents of the body and shell which are accumulated during the first, second and third years, including the reproductive cells which are discharged from the body, it has seemed appropriate to inquire as to the source of the materials from which these constituents have been synthesized. Microscopic and chemical analyses of the feces have been of assistance in supplementing previous observations on the food supply and its assimilation.

Computations have been included relative to available organic matter in the sea as a potential food supply, the average rate of water filtration by the mussels, their approximate rate of oxygen consumption at mean prevailing temperatures and the quantity of fecal material discharged. And finally the influences of the mussel on the biological, physical and chemical properties of the water have been considered, as well as the role of the species as a contributor to the metabolism of the sea.

---

[2] The writers wish to express their appreciation to Mr. John P. Cunningham, who has made most of the chemical analyses, to Mr. David M. Updegraff and Mr. Sheldon C. Crane for assistance throughout this investigation, to Dr. G. F. McEwen for advice on the mathematical formulas, to Mr. W. E. Allen and his associates for quantitative phytoplankton data, and to Dr. C. E. ZoBell for his interest and helpful advice.

RATE OF INCREASE IN SIZE, WEIGHT OF TISSUES, WEIGHT OF SHELL AND AMOUNT OF CALCIUM

With numerous individual exceptions it was found that measurements of the volume of the whole animal with valves closed, weight of tissues and weight of shell bear a logarithmic relationship to length of shell at all ages. Measurements of length, volume and weight follow sigmoid curves when plotted against age, as show in figure 1, while volume and weight yield straight lines when plotted against length on double logarithmic paper, as shown in figure 2. Many individual variations were found in size, in shape of shell, in thickness of shell and in weight of dry tissues, the latter due both to differences in the percentage of water present and in the development of sexual products, as will be explained more fully in subsequent sections of this report.

At the age of about 2 months after metamorphosis, when the length of the shell has reached 10 mm. and the volume of the body 0.15 cc., the dry weight of the tissues is only 0.009 gm. and of the shell only 0.105 gm. During these first 2 months of life the tissues and shell together have accumulated 0.04 gm. of calcium (table 1).

Growth is greatly accelerated during the third month, the length increasing by two-thirds (10 to 17 mm.), while the volume, weight of shell and weight of tissues increase about four fold, or from 0.15 to 0.6 cc., 0.1 to 0.4 gm. and 0.009 to 0.034 gm. respectively.

At the age of 6 months the shell is about 40 mm. long and the volume 5.5 cc. The dry weights of tissues and shell have increased to 0.325 and 3.46 gm. respectively, including 1.36 gm. of total calcium (table 1).

By the end of the first year, when the shell has reached a length of approximately 80 mm., the individual has a volume of 35 cc. The dry tisues then weigh about 2 gm. and the shell approximately 23.5 gm. The total calcium accumulated during the year is about 8.6 gm. (table 1).

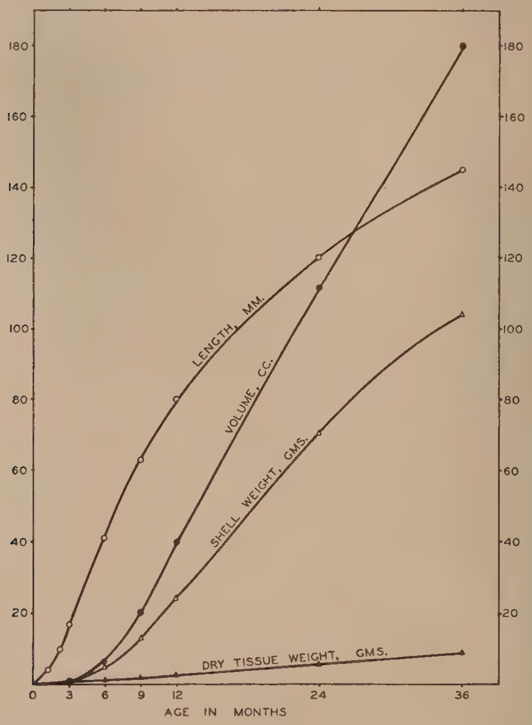


Fig. 1 Graphs showing average increase in length, volume, weight of shell and weight of dry tissues at ages of 3, 6 and 9 months and at 1, 2 and 3 years.

During the second year the absolute monthly increase in volume and weight is greater than in the first year, although the relative rate of increase is less. The increase in length during the second year is only 40 mm. (from 80 to 120 mm.), or half as much as during the first year but the increase in volume is 76 cc., or more than twice as much as in the first year. The dry weight of the tissues is nearly three times as

TABLE 1

Chemical composition of tissues and shells.

Showing increase in length, volume, weight of tissues, weight of shell, percentage of water, of ash and of calcium and the total calcium accumulated at intervals during the first 3 years of life.

LENGTH OF SHELL	VOL.	NO.	TISSUES INCLUDING GONADS				SHELLS			TOTAL CALCIUM
			Water	Dry Wt.	Ash	Calcium	Dry Wt.	Ash	Calcium	
mm.	*cc.*	*Ind.*	%	*Grams*	%	%	*Grams*	%	%	*Grams*
10	0.15	162	77.6	0.009	11.74	1.12	0.105	86.93	37.03	0.04
20	0.8	39	83.3	0.048	14.10	0.44	0.57	92.16	34.70	0.20
30	2.5	19	83.9	0.164	11.30	0.34	1.61	90.06	37.48	0.60
40	5.5	14	85.3	0.325	16.90	0.29	3.46	93.82	39.40	1.36
50	9.0	7	83.1	0.50	13.51	0.24	5.33	95.13	35.01	1.87
60	16	12	81.4	0.93	9.85	0.24	8.24	90.2	37.24	3.07
70	24	9	82.0	1.39	10.14	0.19	13.93	86.24	36.35	5.07
80^{7}	35	5	...	2.00	....	0.20*	23.48	....	36.50*	8.60*
90	48	7	82.9	2.77	10.00	0.19	28.79	92.27	36.65	10.56
100	66	2^{1}	...	3.37	15.50	0.28	....	....	....	....
100	67	1^{2}	...	2.66	16.26	0.23	....	....	....	....
101	68	2^{3}	...	3.52	10.78	0.22	....	....	....	....
110	80	1^{4}	81.6	3.20	7.53	0.20	42.01	93.89	36.80	15.46
120^{8}	111	5	...	5.60	...	0.20*	70.20	....	38.00*	25.91*
125	120	1^{5}	82.5	6.10	9.88	0.20	76.40	....	40.70	31.11
145^{9}	180	1^{6}	85.7	8.07	14.00	0.24	104.50	....	40.20	42.03
185^{10}	410	1	...	...	...	...	202.00	....	....	....

*Estimated. [1] Ripe males. [2] Partially spawned female. [3] Ripe females. [4] Several weeks without food. [5] Nearly ripe female. [6] Nearly ripe male. [7] Age 1 year. [8] Age 2 years. [9] Age 3 years. [10] Age more than 5 years.

great as at the end of the first year and the weight of the shell is also three times as great. Consequently about 17 gm. of calcium are stored in the body during the second year, or approximately twice as much as was accumulated in the first year (table 1).

During the third year the increase in length is only about five-eighths as much as in the second year (120 to 145 mm.) and the volume of the body as well as the weights of tissues and shell increase slightly less rapidly than in the second year (table 1; fig. 1).

Such evidence as is now available indicates that on the coast of Southern California the growth rates of most individuals decrease after the third year, although it seems probable that this conclusion would not apply to more northern parts of the coast where the mussel reaches a much larger size and presumably a greater age.

The rates of increment in length of shell, volume of animal with valves closed, dry weight of shell and dry weight of tissue at the ages of 3, 6 and 9 months and at 1, 2 and 3 years are shown graphically in figure 1. Each graph evidently indicates a portion of a sigmoid curve.

In figure 2 the logarithmic values of volume of whole animal, dry weight of shell and dry weight of tissues for a large number of individuals are plotted against logarithmic values of lengths of shell. With the exception of very large mussels and a few aberrant individuals the measurements for volume and tissue weight lie in approximately straight lines. This is likewise true for the shell weights of individuals less than 2 years of age or 120 mm. in length. In older and larger animals the shell weight increases proportionately more rapidly than the increase in length. This is due to the gradual thickening of the shell by continued additions to the nacreous layer. These variations in weight may amount to more than 25%.

INFLUENCE OF ENVIRONMENTAL CONDITIONS ON RATE OF GROWTH

The experiments on rate of growth of mussels of various sizes as influenced by environmental conditions, reported by Coe and Fox ('42) for the years 1940 and 1941, have been continued during portions of 1942 and 1943. The experimental conditions for the 3 years were similar in that monthly measurements were made on individuals of the same sizes as were used during the first year. This precaution was necessary because it was found that the increment in length is normally more rapid during the third to ninth months of life than at any other period.

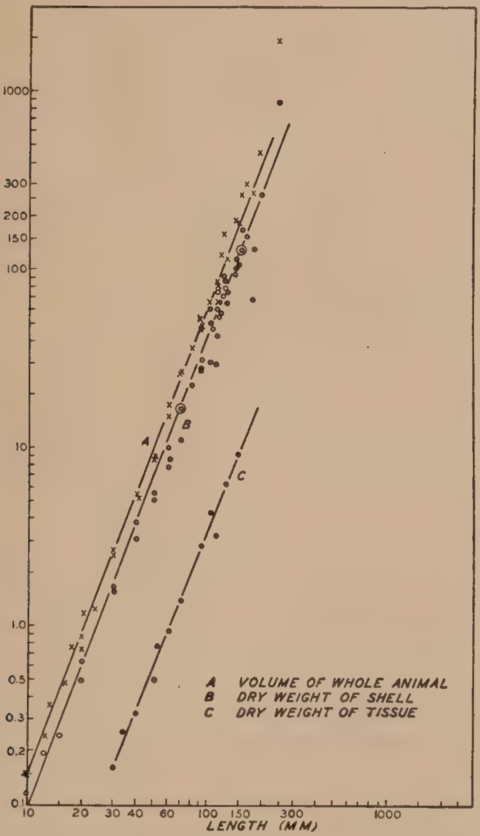


Fig. 2 Comparison of logarithmic values of volume (c.c.), weight of shell (gm.) and weight of dry tissue (gm.) with logarithmic values of length. The close approximation to parallel straight lines is evident for all sizes up to 150 mm. in length. For larger sizes the shells tend to increase in weight more rapidly than in length.

The mean and maximum rates of growth for each month of the 3 years, together with graphs indicating the average numbers of dinoflagellates and the mean temperature of the water for the corresponding periods are shown in figure 3. It will be noted that with the exception of the summer of 1940 and the months of November and December, 1942, and February, 1943, the growth rates for corresponding months of the year show the same general trend.

The increment was generally lowest during the winter, due in part to prevailing storms and heavy surf. There was a rapid rise in the spring and, with the exception of 1940, the rise continued through July. In each year the growth rate decreased during August but in 1940 rose again in September and in 1942 in November (fig. 3).

During the summer months of 1942 the average rate of growth was much greater than at any time in the preceding 2 years. There was a gradual decline until October, followed by an increase in November and December, with a decrease the following month and increases in February and March followed by a rapid increase in May, 1943 (fig. 3).

Food. It is obvious that the rate of growth must be in a large measure dependent upon the food supply. Examination of the contents of the stomach, digestive diverticula and of the feces shows that this species of mussel ingests a great variety of living and dead cells and organic particles, together with such small particles of sand and shells as may be suspended in the water. The ingested material includes dinoflagellates, diatoms, bacteria, flagellates and other protozoans, other microorganisms, invertebrate ova and spermatozoa, unicellular and filamentous algae, algal zoospores, together with detritus consisting of chloroplasts, fragments of cellulose, granules of starch and glycogen, oil globules and protein particles from the disintegrated cells of dead animals and plants (Coe and Fox, '42). In this respect the mussel acts as a scavenger, removing from the water all the particulate matter that enters its incurrent aperture. Large and spiny objects are excluded.

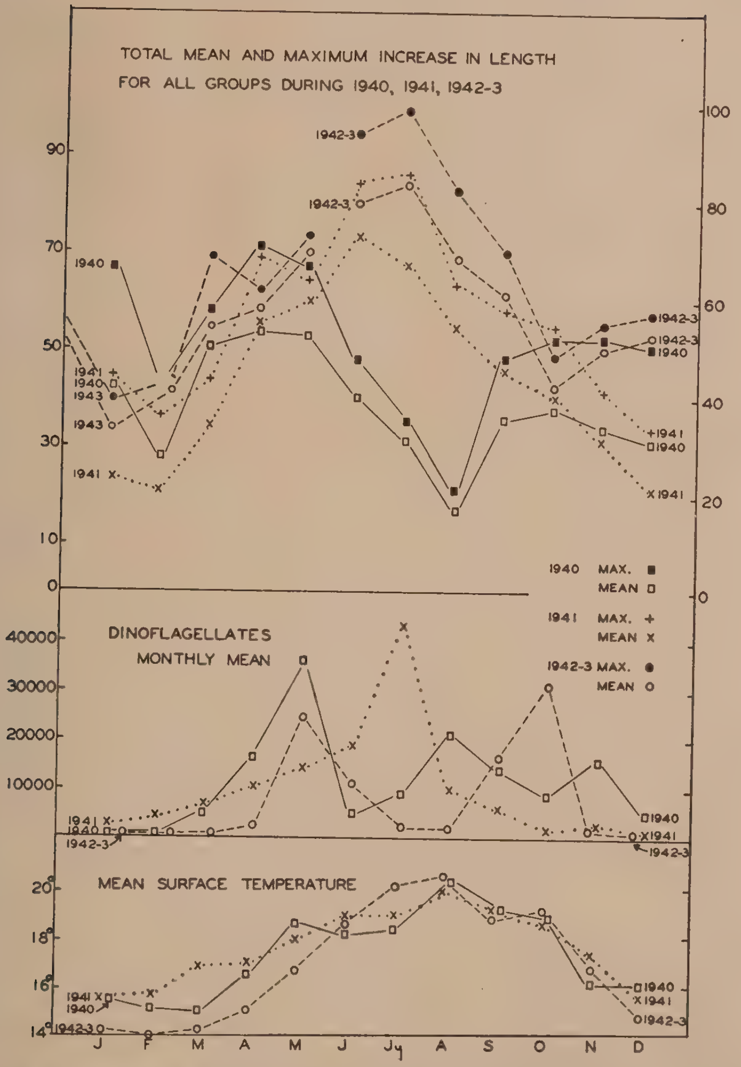


Fig. 3 Comparison of rates of increment in length during the years 1940, 1941, June to December, 1942 and January to May, 1943 with numbers of dinoflagellates and average temperatures during each month. It will be noted that with some exceptions the periods of most rapid growth coincided with large populations of dinoflagellates. There was likewise a generally positive correlation between growth rate and temperature but with a decrease during August, the month with the highest temperature, in each of the 3 years.

The detritus is more important in the nutrition of the mussel than are the living microorganisms.

During the first 2 years there was found a generally positive correlation between the rate of growth and the abundance of dinoflagellates. This was not interpreted as satisfactory evidence that the increase in size was strictly dependent upon the available supply of these organisms but merely indicative that both mussels and dinoflagellates may thrive under the same environmental conditions (Coe and Fox, '42). During the third year the correlation was less evident.

In the year 1940 the most rapid rate of growth occurred in April, when each liter of water contained a daily average of about 16,000 dinoflagellates. In 1941 the maximum rate of growth took place during June and July, the number of dinoflagellates averaging 18,000 per liter in June and 43,000 in July. In 1942 the month of most rapid growth was likewise July, although the water contained only 3,000 dinoflagellates per liter.

Temperature. The graphs in figure 3 also show that, with the exception of the summer months of 1940, there was a generally positive correlation between increment of growth and the average monthly temperature of the water during each of the 3 years, but with a decrease during the month with the highest temperature.

Surf and tides. Mussels exposed to the heavy surf grow more slowly and have thicker shells than those in more sheltered situations. Those living near or below the low-water level grow faster and have thinner and narrower shells than those exposed to the air between tides.

INFLUENCE OF ENVIRONMENTAL CONDITIONS ON SHAPE AND THICKNESS OF SHELL

As is the case with so many other species of pelecypods the environmental conditions under which the mussel grows influence both the shape and the thickness of the shell. These differences are so great that it is often possible to distinguish at a glance individuals taken from the pier from those that have grown upon the neighboring rocks. The former have higher, narrower and thinner shells, with more acute posterior ends than those which have lived under the more crowded conditions on the rock and have been exposed to the greater violence of the surf. There is little doubt that the thicker shells with their blunt edges are indicative of a slower rate of growth. This is further indicated by the fact that individuals from the rocks become sexually mature and spawn at a smaller size than is usual with those living on the pier.

The graph showing the weights of the shells from the two situations (fig. 4) indicates that the two populations represent two morphological phenotypes, for there is only occasional overlapping except in the smaller sizes. The more nearly cylindrical configuration of the shells from the rocks likewise increases both the relative volume of the shell and the relative weight of the tissues as compared with shells of the same length which have grown on the pier.

When these blunt, thick-shelled individuals are placed in the relatively quiet water beneath the pier, and beyond the reach of the surf, the new growth is thin, high and acutely pointed, in conformity with the normal shape of the shells growing at low levels on the pier.

From large numbers of measurements, including the averages shown in table 1, formulas have been derived for approximate volumes and weights of shell and tissues computed from length of shell (L), as follows:

Mixed population from rocks and pier

Volume (V): $\log. V = 2.686 \log. L - 3.56$
Dry weight of shell (W): $\log. W = 2.667 \log. L - 3.699$
Dry weight of tissues (w): $\log. w = 2.541 \log. L - 4.549$

Phenotype from pier

Volume (V): $\log. V = 2.58 \log. L - 3.39$
Dry weight of shell (W): $\log. W = 2.46 \log. L - 3.41$
Dry weight of tissues (w): $\log. w = 2.46 \log. L - 4.41$.

It may be emphasized that the above formulas apply particularly to mussels which have grown on the pier at the Scripps

Institution of Oceanography and on the neighboring rocks, and it is improbable that they will hold precisely for those from any other locality.

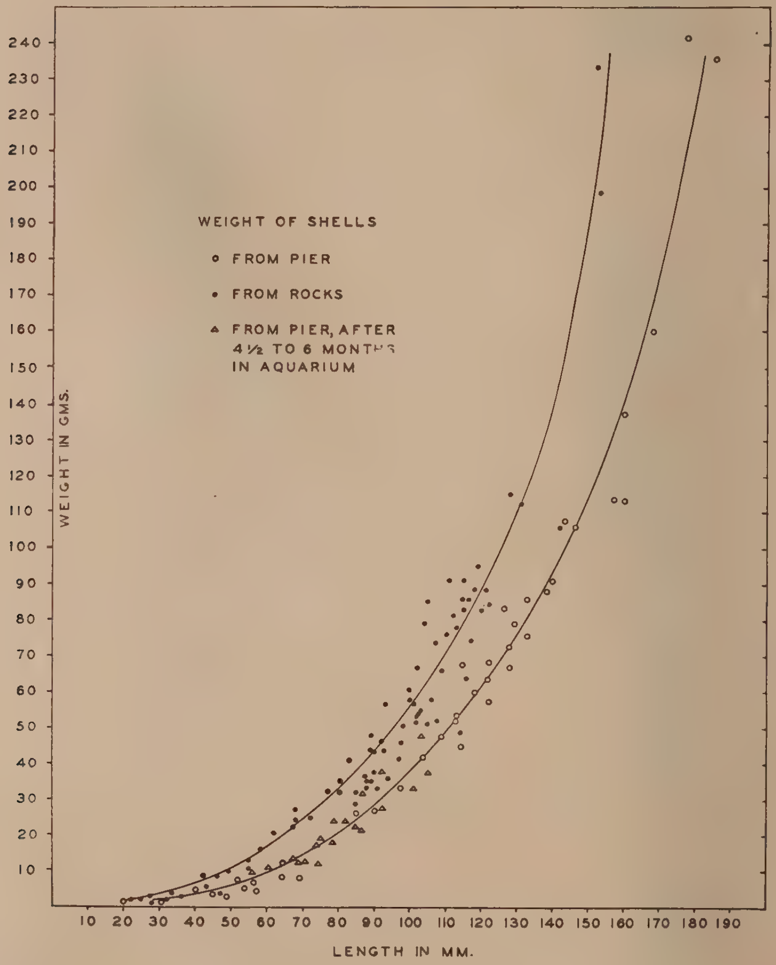


Fig. 4 Graphs showing the effects of environment on weight of shells. The shells of mussels taken from the rocks are so much wider and heavier than those of the same lengths grown upon the adjacent pier that they represent a distinct phenotype. The graphs also show that there is no loss in weight of shells after long starvation in the aquarium.

CHEMICAL COMPOSITION OF TISSUES

The dry weight, ash and calcium content of the tissues and shells of a large number of animals representing a series of size groups from 10 to 145 mm. in length are listed in table 1. These sizes represent individuals of various ages between 2 months and 3 years (Coe and Fox, '42). The number of individuals in each size group varied from 162 for those having a length of 10 mm. to a single one of each of the largest sizes. As has been stated above, an exponential relationship is found between length of shell and volume of whole animal in each age or size group except the older and larger. It was also noted that the logarithmic values of length plotted against the logarithmic values of weight of dried tissues and weight of shell yield nearly straight lines (fig. 2). The actual values for each size group are shown in table 1.

The analyses showed that the percentage of water in the living tissues remains nearly constant at all sizes except the smallest and youngest individuals, in which the tissues contain a somewhat larger proportion of organic matter and less water than in older animals. Approximately 83% of the substance of the normal living tissues consists of water, as found also by Scheer ('40). About the same percentage has been reported for Mytilus edulis (White, '37).

The dry weight of the tissues is therefore closely proportional to the volume of the shell except as influenced by the gonads. The dry tissues usually weigh about one-tenth as much as the shell of the same individual at all sizes up to 100 mm. in length. In animals of larger size the relative weight of the shell increases faster than that of the tissues.

The weight of ash as compared with dry weight of body tissues, including gonads, was determined by fourteen analyses of individuals or groups of individuals bewteen 10 and 145 mm. in length. These analyses would indicate that the ash may vary from 7.53 to 16.90%, with an average of about 11% in whole tissues, and about 10% in gonads alone (tables 1 and 2). The highest percentages of ash shown in these analyses are due to particles of sand and minute fragments of shell which the animals have ingested. Consequently there is a lower percentage of ash after a period of starvation in clean water (tables 1 and 2).

TABLE 2

Relative weights and calcium content of gonads and somatic tissues. Showing weight of body tissues and of gonads before and after spawning, as well as after a long period of starvation, with percentage of ash and of calcium in each.

SEX	LENGTH	SHELL WEIGHT	GONADS			SOMATIC TISSUES		
			Dry Wt.	Ash	Ca.	Dry Wt.	Ash	Ca.
	mm.	*gm.*	*gm.*	%	%	*gm.*	%	%
F[1]	68	12.5	0.70	...	...	0.80	....	...
F[1]	80[5]	23.5	0.88	...	...	1.15	....	...
F[1]	80[5]	21.5	1.80	...	...	1.50	....	...
M[2]	82	24.0	...	...	...	0.45	13.10	0.17
M[2]	86	32.5	...	...	...	0.76	8.10	0.16
F[2]	92	38.0	...	...	...	0.47	10.50	0.36
F[3]	102	53.8	0.42	12.1	0.29	2.31	7.93	0.21
M[2]	113	53.0	0.28	8.75	0.34	2.00	9.61	0.32
M[1]	115	86.0	4.39	...	...	3.16	....	...
M[1]	116[6]	64.0	2.28	11.0	0.26	2.01	9.59	...
F[1]	116	59.1	3.10	...	...	2.96	....	...
F[4]	124	...	2.58	...	...	4.64	....	...
F[1]	124	...	5.90	9.6	0.16	6.40	8.07	0.19
M[4]	129	...	3.15	...	...	6.74	....	...
F[4]	130	98.0	2.36	10.9	0.19	4.40	12.82	0.15
F[4]	132	87.5	3.34	8.0	0.20	5.53	12.44	0.17
M[1]	150[7]	99.8	6.40	...	...	5.70	....	...

[1] Ripe. [2] Starved in aquarium 4½ months. [3] After spawning. [4] Half ripe. [5] Age 1 year. [6] Age 2 years. [7] Age 3 years.

The percentage of calcium in the tissues of small animals appears to be considerably greater than in those of larger size, decreasing regularly in those up to 70 mm. in length and then remaining nearly constant throughout the larger sizes. The tissues of individuals only 10 mm. long have more than three times as large a proportion of this element as was found in animals 30 mm. in length (table 1).

Sexual products

When the gonads are fully ripe both the mesosoma and the mantles are greatly distended with the sexual products. By carefully dissecting the gonads from their surrounding tissues it was found that the dry weight of the gonads may equal or exceed that of all the other tissues of the body combined, but exclusive of the shell.

Only a portion of the gametes are liberated during any single act of spawning, several successive ovulations or discharges of sperm occurring at intervals of several days or several weeks.

It has therefore been impossible to determine the actual weight of the gametes discharged, but this can be estimated with reasonable accuracy by comparing the weights of the gonads in individuals of similar sizes before spawning and after all except the residual gametes have been discharged.

Since the ripe gonads are equal in weight to the somatic tissues, it is estimated that the gonads usually lose more than four-fifths of their substance during the spawning period. This loss doubtless represents the approximate weight of the gametes. Table 2 shows, for example, that one individual had a somatic tissue weight of 2.01 gm. and a gonad weight of 2.28 gm. before spawning, while the somatic tissues of another after spawning weighed 2.31 gm. and the gonad only 0.42 gm. An even greater loss results from long starvation, due to the cytolysis of the supporting tissues.

On the coast of Southern California the first spawning usually occurs at the age of about 1 year. The shell is then approximately 80 mm. in length and the dry weight of the gametes produced is somewhat less than 1 gm. At the end of the second year, when the shell has reached a length of about 120 mm. the mussel may discharge into the surrounding water about 3 gm. (dry weight) of gametes during the spawning period. An individual 3 years old and 145 mm. long may produce more than 5 gm. Consequently, it seems reasonable to assume that each mussel after reaching the age of 2 years may enrich its environment with about 3 gm. of gametes, including 0.006 gm. of calcium annually. At the age of 3 to 4 years this amount may be increased to 5 gm. of gametes, with 0.01 gm. of calcium. In exceptional individuals, the first spawning may occur when the shell does not exceed 32 mm. in length, indicating an age of not more than 6 months.

Hence the total amount of material, exclusive of the shell, which the mussel accumulates during its first year is approximately 2 gm., dry weight, consisting of about 1.1 gm. of somatic tissue and 0.9 gm. gametes. During the second year it adds about 1.8 gm. of somatic tissue, making a total weight of 3 gm., and produces 3 gm. of gametes. Thus the weight of the body before spawning is 6 gm. By the end of the third year it has added 2 more gm. of somatic tissue, making a total of 5 gm., and has formed 5 gm. of gametes. The weight before spawning is 10 gm. (table 2).

It is evident, then, that sufficient food must be taken during the first year to synthesize 2 gm. of organic matter, less 11% of ash. In the second year the amount would be 4.8 gm. (1.8 + 3) and in the third year 7 gm. (2 + 5), in each case less 11% of ash. At the third spawning season therefore the body, exclusive of the shell, loses more than twice as much weight during the discharge of the gametes as it has added to its somatic tissues during the entire year.

SOURCE OF THE NUTRITIVE MATERIALS

It was stated in a preceding section on the influence of environmental conditions that the contents of the stomach and of the phagocytic cells of the digestive diverticula, as well as of the feces, show that the mussel ingests a great variety of cells and particles, both living and dead. These are drawn through the inhalent aperture by ciliary action and are then caught on sheets of mucus and carried to the mouth. All objects and particles suspended in the water, including grains of sand, spicules of sponges and fragments of shells, which are sufficiently small to enter the inhalent aperture are filtered out and carried to the palps at the sides of the mouth. Some are immediately ingested; others, if excessively abundant, are discharged from the mantle cavity as pseudofeces, as is explained more fully in the section on the analyses of feces. Of particular importance in the nutrition of the mussel are the particles of detritus resulting from the disintegration of dead plants and animals.

Daily records are available as to the numbers of diatoms, dinoflagellates and bacteria present in the water surrounding the mussel beds. From these data the possible contribution of each of these constituents of the phytoplankton to the nutrition of the mussel can be computed.

Dinoflagellates. Whenever these organisms are abundant they are ingested in vast numbers. Prorocentrum micans is usually the most numerous representative of the group, although at times the population consists principally of species of Dinophysis, Dinodinium or of Gonyaulax. During the first week in October, 1942, the water contained an average of more than 110,000 of these organisms, principally Prorocentrum, per liter. At that time each 2-year-old mussel presumably took into its mantle cavity about 6,600,000 dinoflagellates each day in the 60 liters of water which it filtered (Fox, Sverdrup and Cunningham, '37). It should not be inferred, however, that all of these were actually ingested; some of them may have been discarded as pseudofeces. Furthermore it is doubtful whether any large proportion of their substance contributed to the nutrition of the mussel, since many of them passed through the digestive tract unchanged, to be discharged in the feces in an apparently living condition. Moreover in spite of their small size they are nevertheless too large to enter the canals of the digestive diverticula in any considerable numbers until after they have become disintegrated.

And even if the mussel were able to utilize the entire substance of the dinoflagellates which are ingested in such large numbers, the total mass of the ingested organisms is insufficient to contribute more than a relatively small part of the mussel's nutrition, as the following computations will show.

Census figures for local mixed dinoflagellate populations for the years 1940, 1941 and 1942 average 11,670, 9,500, and 7,550 cells per liter respectively, giving a general average figure of 9,570.

If we consider Prorocentrum micans as approximating a prolate spheroid of major and minor axes equal respectively to 57 μ and 30 μ and as having a density of 1, then the approximate mass of each cell would be 2×10^{-7} gm. This is equivalent to about 2×10^{-8} gm. dry material per cell. Multiplying this figure by the average number per liter of water and the product by the number of liters passing through the mussel annually gives: $2 \times 10^{-8} \times 9570 \times 22000 = 4.2$ gm. of organic matter from dinoflagellates (comparable in mass to Prorocentrum which is one of the largest local dinoflagellates) available annually to each mussel between its first and second year.

This estimated weight of the calculated 210,540,000 Prorocentrum cells potentially ingested per year per mussel is in good agreement with Brandt's (1898) estimate that from 42,000,000 to 80,000,000 dinoflagellate cells, according to size, are required to yield 1 gm. of organic matter.

Now a mussel reaching the end of its second year (120 mm. in length), increasing its dry weight of organic matter by the observed average amount of 4.3 gm. per annum, would have to utilize with 100% efficiency the entire calculated amount of dinoflagellate material presumably taken in, provided no other food were available. This is manifestly an impossible contingency. It is more likely that the mussel's metabolism operates at an efficiency in the neighborhood of about 10%. Some such conclusion is called for by an inspection of the feces, which often contain great numbers of undigested phytoplankton cells.

Diatoms. The small species are consumed directly and the others constitute a potential source of organic detritus utilizable as food. These were estimated by the settling method as present in the following average populations per liter of sea water at the end of the pier: in 1940, 40,700; in 1941, 17,500; and in 1942, 2,700, yielding an average population of about 20,300 diatoms per liter over the 3-year period. Employing the above average value for the quantity of water filtered by each mussel in a year, we obtain the number of about 450 million diatom cells potentially caught by each mussel per year.

Chemical analyses by Brandt (1898) led him to conclude that 675,000,000 small diatoms are needed to yield 1 gm. of organic matter. On this basis, the estimated 450,000,000 diatom cells available to the mussel each year would contain about 0.67 gm. of organic matter for the consumer's nutrition.

TABLE 3

Statistical constancy of shell weight, associated with great losses in tissue weight during starvation for periods of 4½ to 6 months.

LENGTH	DRY WEIGHT SHELL, GRAMS			DRY WEIGHT TISSUES, GRAMS			
	Starved	Normal (average)	Deviation from average	Starved	Normal (average)	Deviation from average	Per cent loss
mm.							
56	9.8	7.1	+2.7	0.24	0.75	—0.51	68
61	11.2	8.8	+2.4	0.24	0.98	—0.75	75.5
68	13.4	12.8	+0.6	0.28	1.29	—1.01	78
69	12.0	13.4	—1.4	0.26	1.35	—1.09	81
70	12.2	13.9	—1.7	0.23	1.38	—1.15	83
74	17.3	16.3	+1.0	0.68	1.58	—0.90	57
75	11.7	16.9	—5.2	0.39	1.63	—1.24	76
75	18.6	16.9	+1.7	0.70	1.63	—0.93	57
79	18.0	19.4	—1.4	0.56	1.84	—1.28	69.5
79	23.8	19.4	+4.4	0.57	1.84	—1.27	69
82	24.0	21.8	+2.2	0.45	2.04	—1.59	78
85	21.7	24.4	—2.7	0.67	2.25	—1.58	70
86	32.5	25.3	+7.2	0.76	2.32	—1.66	72
87	21.5	26.2	—4.7	0.43	2.39	—1.96	82
92	38.0	31.6	+6.4	0.47	2.79	—2.32	83
93	27.3	32.5	—5.2	0.81	2.87	—2.06	72
101	33.2	39.0	—5.8	0.70	3.47	—2.77	80
103	48.5	41.0	+6.5	1.59	3.65	—2.06	56
105	37.5	43.8	—6.3	1.74	3.83	—2.09	54.5
	Average deviation		+0.036 gm.		Average per cent loss		71.6

Bacteria. Plate-counts of bacteria in local marine waters gave average figures of 276,000 cells per liter in 1940, and 612,000 cells per liter during 1941. Taking the average of these figures, multiplied by a factor of 100 to allow for those organisms which failed to colonize the nutrient media, plus those whose colonies represented not one original cell, but perhaps aggregations of several hundred, we arrive at a value of about 45,000,000 bacteria per liter of sea-water at the site of the mussel beds on the piles of the Institution's pier.

Employing as multiplier another conservative figure, that of 22,000 liters of water filtered per mussel per year, gives a value of about 10^{12} bacterial cells ingested per mussel per year. If we adopt as representative of the average size of a bacterial cell a sphere 1 μ in diameter, each cell should be close to 5×10^{-13} cc. in volume. With a specific gravity of 1, such a sphere would weigh 5×10^{-13} gm., or about 5×10^{-14} gm. when dry. Then 10^{12} cells weighing 5×10^{-14} gm. each would give approximately .05 gm. of dry bacterial substance consumed per mussel per year.

The total available supply of these three constituents of the living phytoplankton would therefore yield only 4.9 gm. of dry organic matter. Of this amount, 4.2 gm. are available from the 220,000,000 dinoflagellates theoretically ingested annually, 0.67 gm. from the diatoms and 0.05 gm. from the bacteria.

Other living cells. The other living cells mentioned in a preceding section as contributing to the nutrition of the mussel must be considered in this connection, although no reliable estimate can be made as to their relative importance. Some of them are present in vast numbers only sporadically, while others occur with more or less regularity throughout the year. At times each liter of water may contain several thousand spores from the giant kelps and other algae. When abundant, these cells are ingested in far larger numbers than can be digested and although they may fill the phagocytic cells of the digestive diverticula there may be several times as many discarded in the feces. At other times the gametes of invertebrates, including those of the mussels themselves, are presumably no less numerous locally.

The importance of the other living constituents of the food is even more difficult to estimate, although it is reasonably certain that they may during certain brief periods supply far more nutritive material than the mussel can possibly assimilate.

Detritus. But all of these possible sources of living material in addition to the phytoplankton, even if fully utilizable, could furnish only a small fraction of the nutriment which the mussel requires in order to build up its tissues and gametes. There is, however, an abundant supply available in the detritus originating from the disintegration of dead plant and animal cells. Moreover the phagocytic cells of the digestive diverticula generally show that particles of detritus form their principal inclusions. Such particles are always abundant in the stomach and in the feces. Some of this detritus has originated in the vicinity and some of it has been brought from many miles distant by means of the ocean currents. Part of it is of terrigenous origin.

These studies offer no evidence that the mussel can assimilate true solutes from the surrounding water. Experiments with other animals however indicate that solutes can be made available only through the agency of various unicellular organisms on which the mussel directly or indirectly feeds.

Bond ('33), for example, estimated that the water in East Sound, State of Washington, contained 3.3 times as much "dissolved" as particulate organic matter in the summer of 1931. He concluded from his experiments that there is no evidence that any multicellular animal is able to utilize this vast store of material until it has been synthesized into particulate form by bacteria and other unicellular organisms. Krogh ('34) estimated that the quantities of unorganized organic matter in Bermuda waters exceed the total organic dry substance from living organisms by 300-fold. The reader is not informed by either author exactly what is meant by the term "dissolved." Juday ('42) and others, however, define as "dissolved organic matter" in fresh-water lakes not only organic substances in true solution, but also finely divided, noncentrifugable organic detritus, partly in a colloidal state, and even small bacteria as well. Figures given for such material are of the order of 8.4 to 12.5 mg. (dry weight) per liter, exceeding the weight of total plankton by from 6- to 9-fold.

It is evident that the products of cellular disintegration range in size from large fragments of the cell or entire cell membranes, chloroplasts, starch or glycogen granules and the like through increasingly smaller but still discrete particles of protein, lipoid or carbohydrate to substances of colloidal status and eventually to those capable of existing as true solutes. The exact state of fineness at which the mussel is unable to avail itself of these substances has not been established. Particles the size of small bacteria have been shown to be ingested and assimilated by the mussel, as reflected by its growth (ZoBell and Feltham, '38). Preliminary experiments by us have also demonstrated the mussel's ability to remove from its medium very fine, uncentrifugable colloidal material such as Congo Red or boiled "soluble starch" in highly dilute dispersions, ingesting some of this material and expelling the rest as pseudofeces.

Krogh and Keys ('34) arrived at no definite conclusions regarding the quantitative ratio between organic matter present in the sea as true solutes and that existing in a finely particulate or colloidal state. Nor are we aware of any data bearing upon this important consideration. But in the case of blood, 99% or more of the total organic matter is in a colloidal state, rather than in true solution. Therefore it would seem highly probable that the greater portions of all cell contents set free in the water enter the medium in a particulate or colloidal state rather than as true solutes. We consider as an important source of the mussel's food this kind of marine detritus, existing in finely divided, partly even colloidal condition. To be rendered utilizable by a multicellular organism such as the mussel, organic solutes, e.g., amino and other organic acids, monosaccharides, and dissolved nitrogenous waste products, must first be converted into particulate matter by bacteria or other unicellular organisms, which assimilate the raw materials into protoplasm.

ZoBell and Grant ('42, '43) have demonstrated the capacity of marine bacteria to multiply while utilizing quantitatively all glucose or peptone at concentrations as low as 1 part in 10 million. They conclude that such microorganisms may assimilate these or similar substrates at even lower concentrations. In such considerations, the importance of adsorbing surfaces, whether living or inanimate, must not be forgotten. Doubtless many organic solutes are utilized directly by bacteria present at the former's very sources; bacteria, aggregated upon large masses or minute particles of utilizable detrital substance, may be viewed both as the lytic agents responsible for the derivation of organic solutes from such material and as the chief consumers of such products in the perpetuation of their own species.

Krogh ('34) provides us with figures of 1.5 mg. "protein" and 3.9 mg. carbohydrate per liter in waters off Bermuda. This total average quantity of 5.4 mg. organic matter per liter is in good agreement with the average figure of 5 mg. per liter found by Dr. ZoBell and his associates at La Jolla.

In constructing tables 4 and 5, we have treated the estimated 5 mg. of organic matter per liter of water as though it were all particulate, and as such, adsorbable by the mucus sheet secreted by the mussel at the time of feeding. It will be noted, however, (table 5) that a considerable excess, amounting to nearly 39%, is calculated as unaccounted for. This excess may be assumed to exist in small part as solutes, hence unavailable to the mussel, but chiefly as material which, because of an excessive supply or its refractory nature, may have been discarded as pseudofeces. In any event, wastefulness is a prominent characteristic of the water-filtering mussel.

Metabolism. Studies of the rate of O_2 consumption and CO_2 production by Mytilus californianus, carried out by Whedon and Sommer ('38), provide a useful average figure of 1.58 cc. O_2 per 10 gm. wet tissue (or 0.79 cc. per dry gram) per hour by mussels comparable to our animals in size. The temperature of 17.2°C. employed by Whedon and Sommer was the average prevailing sea-temperature over the 3 years during which our own investigations were carried out at La Jolla. Their studies of the mussel's respiratory quotient reveal a grand average value of 1.21. While they suggest that a part of the CO_2 may arise through the buffering action of the calcareous shell, it is nevertheless probable that CO_2 from that source reflects the production of an acidic catabolite by the animal.

Applying the above data to our studies, one of our mussels would probably consume, between its first and second years (average dry weight 3.9 gm.), some 26,300 cc. or 38 gm. of O_2, producing approximately 31,800 cc., or 62.5 gm. of CO_2, while an animal passing from its second to its third year (average dry weight 7.3 gm.) would consume during the interval about 50,600 cc., or 73 gm. of O_2, evolving some 61,300 cc., or about 120 gm. of CO_2.

TABLE 4

Provisional metabolic balance-sheet of the individual mussel, expressed in grams per year.

AGE-SPAN IN YEARS	ESTIMATED AMT. ORGANIC MATTER ENTERING MANTLE CAVITY	NEW SOMATIC TISSUE FORMED (DRY, ASH-FREE)	NEW GAMETE-TISSUE FORMED (DRY, ASH-FREE)	ESTIMATED FECES VOIDED (DRY, ASH-FREE)	ESTIMATED O_2 CONSUMED	ESTIMATED EXCESS MATERIAL (DRY WT.)	ESTIMATED CO_2 GENERATED	CO_2 LAID DOWN IN NEW SHELL (PROPORTION OF TOTAL)
0 to 1st	50	1.1	0.7	10	10 (equiv. to 10 gm. org. matter)	28	16.5	10 (60%)
1st to 2nd	110	1.6	2.7	26	38 (equiv. to 38 gm. org. matter)	42	62.5	20 (32%)
2nd to 3rd	150	1.8	4.5	34	72.5 (equiv. to 72.5 gm. org. matter)	37	120	19 (16%)

Based upon what we believe to be conservative figures for all of the estimated quantities involved, a portion of the mussel's "metabolic balance sheet" is summarized in table 4.

In the work of Fox, Sverdrup and Cunningham ('37) no data are presented for the rate of water-propulsion by a 3-year-old mussel (145 mm. long). However, the nearly linear relationship existing between weight of feces deposited and age (length) of mussels of 1, 2 and 3 years, together with interpolated values for rate of water-propulsion by small, medium-sized and large animals have provided us with the conservatively estimated value of 3.5 liters of water filtered per hour by an animal growing from its second to third year, as compared with 2.6 liters for a 1- to 2-year-old animal.

Weight of potential food material available per year is calculated on a basis of the approximate figure of 5 mg. per liter, multiplied by the quantity of water filtered by each mussel per year. For the two larger sizes the respective values of 30,000 and 22,000 liters have been retained. For mussels reaching the length of 80 mm. at the end of their first year, the conservative figure of 10,000 liters per year has been employed (Fox, Sverdrup and Cunningham, '37).

Weights of tissue are average values from the results of many measurements. Weights of feces voided per year are based, for purposes of approximation, on the single series of measurements after 24 hours in the sea at a low dinoflagellate population-level, as mentioned in the following section. Values are based upon the average rates at the beginning and at the end of the growth-year.

The quantity of food material in excess per year (i.e., unaccounted for) is always a fairly large figure, obtained merely by subtracting from the estimated potential supply in the water, the sum of the three factors: weights of added tissue and gametes, weights of voided feces, and calculated weight of food oxidized, adopting the conservative value of 1 mg. per mg. O_2 consumed in this carbohydrate feeder (e.g., 192 mg. O_2 completely oxidize 180 mg. glucose).

Oxygen supply and carbon dioxide generation are calculated from the data of Whedon and Sommer, and are applied by us, for purposes of rough generalization, to mussels of all ages considered.

Finally, quantities of carbon dioxide laid down as shell are calculated on a basis of weights of shell and chemical analyses of the latter, the CO_2 content being close to 40% of the shell material.

If, with the measurements and estimations now at our command, we should consider a special population of mussels including 50% in their first year (0 to 80 mm.), 30% in their second (81 to 120 mm.), and 20% in their third (121 to 145 mm.), some simple calculations lead to an interesting general aspect of local alterations in the sea effected by the mussel.

TABLE 5

Biological and chemical exchanges between the mussel and the sea, estimated in grams per year per 100 mussels and per kilogram of shell material.

	CONSUMED		RETURNED			FIXED		
	Potential food: *detritus* + dinoflagellates, bacteria, diatoms, larvae, gametes, etc.	O_2	Feces (dry, ash-free)	Gametes (dry, ash-free)	CO_2	Somatic tissue (dry, ash-free)	New shell	EXCESS UNACCOUNTED FOR
Per 100 mussels of designated distribution	8800	3090 (equiv. to 3090 gm. org. matter)	1960	206	5100	139	3610 (≏ 1444 gm. or 28% of total CO_2)	3400
Per kg. of shell from same 100 mussels	1520	532 (equiv. to 532 gm. org. matter)	337	35.5	880	24	622 (≏ 249 gm. or 28% of total CO_2)	590

Employing weighted means, we may estimate the quantity of materials taken from the sea, the amounts fixed by the mussels, and the material returned to the sea per 100 animals or per kilogram shell formed per year. One hundred mussels distributed according to the proportions above would possess about 5.8 kg. of shell. These estimates of the mussel's biochemical contributions to the metabolism of the sea are shown in table 5. It is evident that the mussel may burn about 35%

of the organic matter theoretically consumable, fixing some 4% into somatic and germ-cell protoplasm, leaving 61% unutilized.

The attainment of greater size by this species in colder waters is presumably due to the retardation in metabolic oxidation, with corresponding increase in rate of storage at lower temperatures, as well as a greater length of life.

Digestion. Brandt (1898) estimated the relative amount of protein in the ash-free dried substance of dinoflagellates as 13.7% and of diatoms as 28.7%; fat 1.37 and 8% respectively; and carbohydrate 84.9 and 63.2% respectively. Yonge ('26) concluded that the carbohydrates are the most important constituents of the food of the oyster; the proteins were thought to be always in excess of the requirements. He found only amylase-like enzymes in the lumen of the digestive tract. The fats and proteins are digested intracellularly by the cells of the digestive diverticula. Phagocytes in the lumen of the stomach or gut may also ingest small organisms or other food particles and then migrate into the epithelium and complete their digestion. The glycogen and fat of both the oyster and the mussel are stored in the vesicular connective tissues.

Fox et al. ('36) likewise found only amylase and glycogenase in significant amounts in the style of the digestive tract of the mussel, while the intracellular enzymes consist of the same polysaccharidases as well as disaccharidases, proteases and lipases.

COMPOSITION OF FECES

Since it is impossible to determine accurately the amount of food which the mussel normally ingests, some evidence as to the portion utilized may be obtained from the examination of the feces. As previously described by Dodgson ('28) for Mytilus edulis and by Fox et al. ('36) for M. californianus, mussels taken from the sea usually begin the egestion of grayish brown, straplike ribbons of fecal material very soon after being again placed in sea water. The substance of these ribbons consists partly of colorless particles of organic and inorganic detritus and the empty tests of dinoflagellates and diatoms; it may also contain many undigested or partially digested dinoflagellates and diatoms, as well as plant plastids, algal cells, bacteria and other particles resulting from the disintegration of plant and animal cells. Some of these minute organisms which have thus passed through the digestive tract appear to be perfectly normal and indistinguishable from those living in the sea. Others show various effects of the digestive enzymes.

It is evident that when food materials are abundant, the mussel often ingests a much larger amount than can be assimilated or even digested during the passage through the alimentary canal. Examination of the living cells of the digestive diverticula indicates that phygocytosis and intracellular digestion of the food particles are responsible for the greater portion of the animal's nutrition.

The constituents of the feces will obviously depend mainly on the character of the food available at the time of ingestion. When dinoflagellates are abundant the remains of these organisms will constitute the principal portion of the fecal ribbon but when the phytoplankton consists mainly of small diatoms the feces will be correspondingly changed. The feces indicate that at all times a great variety of cells of plant and animal origin and much detritus have been utilized. When algal zoospores are abundant the phygocytic cells of the digestive diverticula are distended with these reproductive cells. At such times they also form the principal constituent of the feces, since the mussel is able to assimilate only a small proportion of the vast numbers ingested. Many species of algae contribute their quota of zoospores at various periods during the year.

The abundant and varied food supply throughout the year on the coast of Southern California allows the mussel to continue its growth at all seasons but at an increasing rate in spring and summer when, as a general rule, the minute phytoplankton is most abundant (fig. 3).

After the fecal ribbons and pseudofeces have been broken up and their contained particles are again suspended in the water, they may again serve as food for the same or other animals which then devour and complete the digestion of the nutritive materials which were formerly so wastefully discarded.

In addition to the organic substances mentioned, the feces always contain many minute particles of shell, much sand, mud and other inert detritus.

Whenever the water contains particles small enough to enter the inhalent aperture but too large or too numerous to enter the mouth or which are mechanically or otherwise objectionable, they are segregated by the labial palps, imbedded in mucus and extruded as cords of pseudofeces. Such cords may be very abundant when the animal is kept in water containing much suspended matter, such as mud or minute particles of sand or shells, as well as when large numbers of microorganisms or organic detritus particles are present. Minute ova, larvae and other cells in such pseudofeces are usually returned to the sea while still in a living condition. The pseudofeces are extruded from the mantle cavity along the ventral surface of the branchial membrane and not from the exhalent aperture as are the true feces.

The amount of feces voided will obviously depend upon the food supply and is therefore subject to great variation. During the first 3 days after well-nourished mussels are brought into the aquarium from the sea, feces are discharged at a nearly constant rate, the amount from each animal being approximately proportional to its size.

Series of ten animals in each of five sizes were taken from the sea at a time when the water contained about 3000 (a relatively low level) dinoflagellates and diatoms per liter, and placed in running sea water. At the end of 24 hours the feces were collected from each segregated lot, twice decanted from the sand which is an invariable constituent, and finally dried in a vacuum at room temperature and weighed, with the following results: animals of 20, 40, 60, 80, and 120 mm. in length discharged respectively 0.0075, 0.045, 0.095, 0.176 and 0.281 gm. of feces per mussel.

After the fourth day in the aquarium the amount of feces decreases rapidly unless the aquarium water contains more than its usual supply of food materials. The percentage of chlorophyll-bearing cells likewise decreases.

Determination of ash, organic matter and calcium. The feces from about twenty large mussels were collected daily for the first 7 days following the animal's transfer from the sea to the aquarium. As much sand and shell as possible was separated by comminution and subsequent decantation from the samples at each collection.

The feces voided during the first 3 days were combined in one lot (A) and those collected during the succeeding 4 days in a second lot (B). The following analyses were made:

			A	B
N	(dry wt.)	(organic by Kjeldahl)	1.02%	1.06%
PO_4	(dry wt.)		0.31	0.60
Ca	(dry wt.)		3.03	2.75
Mg	(dry wt.)		0.50	0.59
Total ash	(chiefly sand and shell)		63.8	69.34

Two additional samples from other specimens collected at a different time contained 74.3% and 68.87% ash, respectively, 4.48% and 16.56% Ca (the latter high value being presumably due to an unusually high proportion of fine particles of shell), 0.6% and 0.7% PO_4, and 1.19% and 1.13% N.

Multiplying nitrogen values by the factor 6.25, commonly accepted as applying to the proteins of marine origin, we obtain about 6.5% as an average protein content of series A and B, and 7.25% as an average of the two other samples.

The chemical analyses are therefore in harmony with the microscopic examinations in showing the wastefulness of the digestive apparatus when food is abundant.

EFFECTS OF STARVATION

The water supplied to the aquarium at the Scripps Institution of Oceanography does not contain a sufficient supply of microorganisms and particulate organic matter to enable the mussel to survive for more than 6 to 10 months at temperatures of 14° to 21°C., which are seasonally normal for this locality. Large individuals with full gonads live longer than others and survival is longest during the cooler portions of the year. Increase in size occurs only when supplementary food is provided (Coe and Fox ,'42), either directly or incidental to the presence of algae, fish or other organisms.

After 4½ to 6 months in the aquarium the body tissues become thin and watery and lose from 54.5 to more than 83% of their dry weight (table 3). The gonads, unless nearly ripe, undergo cytolysis and most of the ova or spermatozoa previously formed, as well as the ovogenic or spermatogenic cells, are utilized in supplying energy for the essential metabolic processes. In both sexes the unripe gonads are eventually converted into a loose connective tissue framework with follicle cells containing reddish granules and aggregations of minute vacuoles as disintegration products.

If the gonads are nearly ripe at the beginning of the fasting period the mature gametes may be spawned, sometimes after several months (Scheer, '40).

To determine whether there is any loss in the weight of the shells in animals subjected to long starvation, the shells of nineteen individuals which had been in the aquarium during 4½ to 6 months were carefully cleaned and dried. Ten were found to weigh more than the average previously found for shells of the same lengths under normal conditions and nine weighed less than the normal average (table 3). Of those with more than average weight the average excess was 3.51 gm., while those of less than average weight showed an average weight of 3.82 gm. less than the normal average for the same sizes (table 3). The average for the entire group was 0.036 gm. above the normal average.

These figures indicate that any changes in shell weight during the maximum period of starvation are too small to be statistically significant. Nevertheless, a very slight loss of weight is theoretically possible under certain conditions, since in some mollusks it is known that the mantle is able to dissolve shell as well as to deposit it. Kühnelt ('38) found that a very thin layer from the inner surface of the shells of various mollusks was dissolved by the accumulated CO_2 whenever respiration was inhibited for even a few hours.

CHEMICAL COMPOSITION OF SHELLS

The dry weight of the shells, as shown in table 1, indicates an exponential increase in weight with arithmetical increase in length. The ratio of weight to volume tends to decrease slightly as the length increases during the first 2 years of life. Thereafter the weight increases more rapidly than the volume. There is, however, much individual variation in the thickness as well as in the shape of shells produced under different environmental conditions. It was mentioned in a preceding section that the mussels living on the pier have much thinner shells than those of the same length growing on the neighboring rocks.

The percentage of ash in the shells varies considerably with age and environmental conditions. The shells of young individuals 10 mm. in length have less than 87% inorganic material, while those of larger sizes usually have from 90 to 95% (table 1). This would indicate an average of approximately 8% of organic matter. This is also the percentage usually reported for Mytilus edulis, although values relating to the latter species have been found to vary according to the salinity of the water.

The percentage of calcium in the shells varies from 34.7 to 40.7%, with an average of about 37%, corresponding to about 92.5% $CaCO_3$. This is approximately the average which Clarke and Wheeler ('22) found in the shells of other species of pelecypods. The highest calcium content occurs in the shells of the older and larger individuals. The percentage of calcium is also higher in the relatively thick shells taken from the rocks than in the thinner shells secreted by individuals growing on the neighboring pier.

The total amount of calcium in the whole animal, including both tissues and shell, increases from 0.04 gm. in the 10 mm.

individual to more than 42 gm. in an animal 3 years of age (table 1).

If the length of the mussel at the age of 1 year is about 80 mm. and its volume 35 cc., the weight of the shell would average between 23 and 24 gm. The analyses show that the body tissues at that time contain only about 0.2% calcium, while the shell is composed of more than 36% of this element. Consequently a normal mussel will have stored in its body and shell some 8.6 gm. of calcium by the time that it is 1 year old (table 1).

During the mussel's second year the length of the shell becomes increased to 120 mm. and its weight to 70 gm., representing about 26 gm. of calcium. This is an increase of more than 17 gm. of calcium during the year. At the end of its third year the shell has normally attained a length of 145 mm. and a weight of 104 gm. or more, of which 42 gm. are represented by calcium. Thus the increase in calcium during the third year is 16 gm. (table 1).

The average monthly storage of calcium would therefore be about 0.7 gm. during the first year of life, 1.5 gm. for the second year and 1.34 gm. for the third year.

The increase in shell weight and correspondingly the increase in total calcium is considerably less in the mussel than in bivalves with thicker shells. The Virginia oyster, for example, may increase its shell weight by as much as 75 gm. in 1 year (Galtsoff, '34), as compared with a maximum of less than 50 gm. for the mussel in this locality.

SOURCE OF CALCIUM

The question now arises as to the source of all this calcium — whether it is wholly supplied by the food and other ingested material, including particles of shell, or whether some of it is taken directly from the sea water in soluble form. The question can be answered at present only from circumstantial evidence.

If the dinoflagellates be selected as an example of the food supply and if these organisms contain about 0.43% of calcium, as has been determined by the calcium-nitrogen ratio of various analyses, it may be calculated that the mussel may theoretically obtain approximately 1 gm. of calcium from each 1,500,000,000 dinoflagellates. Assuming an intake of 250,000 per day or 91,250,000 per year, these organisms would supply a 100 mm. mussel with only 0.062 gm. calcium in a year. The organic matter in the diatoms and other organisms consumed, including detritus, would contain only a fraction of a gram more. It was shown in the preceding section, however, that a mussel of the size mentioned normally accumulates in its tissues and shell some 17 gm. of this element during that period of time, or while its length is increasing from 80 to 120 mm. (table 1). To supply this amount of calcium would require the consumption of no less than 4200 gm. of food containing 0.4% calcium during the year. This would be at the rate of 11.5 gm. per day, or many times the amount of particulate organic matter available in all the water which passes over the gills.

It is obvious therefore that the greater proportion of the calcium must be taken directly from the water, either in solution or in particulate form. Fox, Sverdrup, and Cunningham ('37) have shown that the mussel may pass water through its gills at an average rate of 2.6 liters per hour, or 22,000 liters in a year, provided filtration continues incesantly. Such a volume of water contains about 9240 gm. of dissolved and colloidal calcium, and it would therefore be necessary for the mussel to utilize only about $\frac{1}{5}$ of 1% of this amount in order to obtain the required 17 gm. from this source alone.

There is satisfactory evidence that the Virginia oyster obtains much of its calcium in this manner, for Galtsoff ('34) has shown that the deposition of shell continues at an undiminished rate during the months when no food is taken. The amount of calcium thus utilized is many times greater than could have been stored in the tissues. Furthermore, the tissues do not decrease in weight. In the English oyster (Ostrea edulis) likewise the shell continues to grow in the absence of food (Orton, '25).

In the California mussel, on the contrary, no appreciable increase in size occurs unless food is supplied (Coe and Fox, '42), but this fact does not necessarily imply that the calcium is obtained from the food. It only indicates that a continuous food supply is essential for the normal utilization of the calcium from whatever source derived.

A third source of calcium consists of minute particles of the disintegrated shells of this and other species of mollusks, barnacles, calcareous tubes of annelids and calcareous algae. These are always present in the water surrounding the mussel beds and they are invariably found in the digestive tracts of actively feeding mussels. They are also constantly present in the feces. It is not improbable that a considerable portion, and perhaps the major portion of the calcium utilized in the secretion of the shell is obtained in this manner. The lumen of the stomach has a pH of 5.92 to 5.95 and is therefore sufficiently acidic to effect the solution of some of the calcareous particles. Other particles may be dissolved by the cells of the digestive diverticula, which have a pH of 6.03.[3] The calcium ions may thus reach the blood and be carried thence to the secreting cells of the mantle, whose freshly exposed outer surface shows pH values of 7 or slightly above, favoring precipitation of $CaCO_3$.

It has long been known that the shells of fresh-water mollusks become thicker in situations where the water contains a large amount of calcium but it has not been proved whether the calcium used in secreting the shells is obtained from the food or taken directly from the water. Experiments have shown that the addition of powdered calcium carbonate to the diet of terrestrial gastropods greatly increases the thickness of their shells. It is well known that snails will commonly rasp off any available pieces of chalk.

Since preliminary experiments by Robertson ('41) failed to indicate whether certain marine gastropods and pelecypods are able to take calcium ions directly from sea water, experiments were devised in the hope of obtaining evidence as to the source of the calcium used by the mussel in forming the shell.

---

[3] Determinations of pH were made with the Beckman glass electrode and pH meter.

Feeding experiments

Scheer ('40) found that mussels of this species kept in vessels of aerated water and supplied with a variety of artificial diets, including one group fed on a pure culture of the diatom Nitzschia, invariably showed a loss of tissue weight after a month or more. The loss was smallest in the group fed on Nitzschia.

In an attempt to ascertain whether the mussel might assimilate its calcium supply directly through the digestive tract by ingestion of calcareous materials, three series of feeding experiments were conducted, using animals of a size which show rapid growth under natural conditions.

Experiment 1. Twelve individuals, having an average length of 29.5 mm., were placed in each of two battery-jars containing 9 liters of sea water. Marble chips were added to each jar to aid in maintaining a normal pH value and to keep the water near saturation with $CaCO_3$. The water was aerated continuously, kept in a dark room at a temperature of 16.5°C., changed every few days, and maintained at a constant volume by the addition of distilled water when necessary.

Each group of animals received a diet of Ulva which had been oven-dried at 110°C, finely ground in a ball mill and stored in tubes containing 0.2 gm. dry weight of Ulva and about 10 ml. of sea water. The tubes were plugged with cotton and sterilized by autoclaving.

Both groups were fed the contents of one of these tubes daily for 12 days. After the animals had filtered the food from the water, group 2 received 2 gm. of powdered $CaCO_3$. Group 1 received only the basic diet of Ulva. The formation of pseudofeces indicated that both groups were receiving all and more material than they could ingest, while the voiding of true feces showed that part of the material had passed through the digestive tracts. Since measurements of the animals after a 12-day period on 0.2 gm. of Ulva per day showed no perceptible increase in size, the amount of Ulva was increased to 0.4 gm. daily. No increase in size occurred during 4 weeks.

The diet was then changed to 0.4 gm. of finely-ground corn meal per day, prepared and administered in the same way as the Ulva. Since this gave no growth after 16 days the diet was again changed to 0.2 gm. of Ulva, 0.2 gm. of corn meal and 0.1 gm. of dried mussel flesh daily for 1 week. No growth occurred.

In order to test the hypothesis that the heating and drying of the food might have destroyed necessary growth-promoting substances, the same mussels were later fed for 3 weeks on a fresh finely-chopped eel grass and kelp mixture in amounts of 0.1 to 0.2 gm. dry weight per day. There was still no growth.

At the end of the third month, not a single one of the mussels in either group had increased appreciably in length. In both groups there was a loss in tissue weight amounting to about 6%. In both groups the weight of the shells remained constant. A group of control mussels of the same size living in the sea had each gained about 20 mm. in length during the same period.

Experiment 2. In a second experiment, group 3 (eleven animals, average length 31.0 mm.) received food only; group 4 (eleven animals, average length 30.25 mm.) received the same food plus calcium carbonate, while group 5 (eleven animals, average length 33.37 mm.) received neither food nor calcium carbonate. During the first 5 weeks, the fed groups received mixed fresh, finely-ground eel-grass (Phyllospadix) and Eisenia arborea, a brown kelp, in amounts of about 0.2 gm. daily. Observing no increment in length after this time, the former diet was replaced by 1 gm. ground mussel feces daily for another month.

In this second experiment no increase in length occurred and the losses in weight were even greater than in the first, amounting to 47.2% in tissue weight for group 3; 51.7% in tissue weight for group 4; and 42.4% in tissue weight for group 5. There was no change in shell weight.

It should be noted that the control group 5, which was kept in aerated water only, without food or powdered calcium carbonate, sustained smaller losses in tissue weight than either of the other groups. This fact may indicate that in the latter not only was there a failure to utilize the materials supplied as food, but that from them decomposition products were formed which were actually harmful.

Experiment 3. An earlier exploratory experiment was more successful insofar as growth was concerned. Here the mussels were fed each morning with mixed cultures of diatoms, scrapings from the surface of mud, containing algae and associated microscopic organisms and detritus, with occasional additions of living spermatozoa, crushed ova and ground desiccated annelid and mussel tissues. Each afternoon the water containing the uningested portions of these food materials was replaced by fresh sea water. The mussels were consequently able to feed only about 8 hours daily, but a greatly excessive and varied diet was provided. Because of frequent changes of water there was no accumulation of decomposition products. In this experiment, extending through 3 months, there was an average monthly increment in length of about 2 mm. This was approximately one-third as great an increase as was observed in animals of similar sizes living in the sea and which consequently were able to feed three times as long each day (Coe and Fox, '42) during the same period. In a control group, without artificial feeding, there was no perceptible growth. This experiment, however, provided no indication as to the source of the calcium utilized.

The evidence at present available leads to the conclusion that the mussel, in common with other marine bivalves, depends upon the calcium compounds in the sea water to supplement those contained in the organic constituents of the food in supplying the materials necessary for the secretion of the shell. Whether the calcium is taken in colloidal or particulate form, or in true solution is not yet known. There is no evidence that this element absorbed otherwise than through the digestive tract, but further experimentation along this line of investigation is in progress.

INFLUENCE OF THE MUSSEL ON THE CHEMICAL AND PHYSICAL PROPERTIES OF THE SEA WATER

Physical effects. The physical changes which the mussel Mytilus edulis produces in the sea water as the result of its filtration activities have been mentioned by numerous observers. The importance of mussels as agencies for removing pathogenic bacteria and other microorganisms, as well as all other suspended particles, from the sea has been the subject of extended experimental studies by Dodgson ('28). All the suspended particles which enter the inhalent orifice of the mussel are arrested by the gills, imbedded in mucus and then either taken into the mouth or discharged from the mantle cavity as pseudofeces. Many of the bacteria which enter the mouth are assimilated by the digestive system. Those that are first discharged in the pseudofeces may be again taken into the body and thence to the mouth. Mussels from a contaminated locality may temporarily harbor in their digestive systems large numbers of pathogenic organisms and thus become a menace to health if eaten without suitable cooking. But if such mussels be kept for a few days in a closed tank of sterilized sea water the bacteria will all be digested and the mussels will be fit for human consumption (Dodgson, '28).

The rate of filtration in the California mussel and the importance of this species in removing suspended mud and other matter from the water has been reported by Fox, Sverdrup and Cunningham ('37). Experimental animals having a shell length of 95 to 130 mm. were found to filter an average of 2.6 liters per hour.

All suspended matter which enters the inhalent orifice of the mussel is removed as the water passes over the gills. The greater part of it is soon returned to the sea as feces or pseudofeces but a small proportion is assimilated. Most of the elements composing the assimilated material are returned later as gametes or excreted in solutions; only a small proportion is retained for the upbuilding of the body and shell.

This physical influence of the mussel on the surrounding water is described by Fox et al. ('36). "Experiments with muds, carborundum, charcoal, certain insoluble metallic compounds, and other indigestible materials indicate that the mussel will consume large quantities of nutritionally useless matter which is nontoxic, but will reject poisonous material. In nature, mussels may effect considerable alterations in the physical properties of sea water in certain localities, both by swallowing indigestible detritus (such as fine silt, mud or clay) and subsequently expelling it in the form of fecal pellets, and by incorporating much of the material in ropy, mucous strands and rejecting it as pseudofecal matter, emitting small particles from the exhalent siphon and larger ones from the edge of the mantle. This kind of agency at work near river mouths or regions of copious run-off might, under certain conditions, greatly alter the nature of both the water and the bottom, not only at the site, but also (probably indirectly) at some distance out from shore."

Biological effects. The ecological relationships which exist between the mussels and the other animals and plants in their vicinity relate to the organisms which are secured as food and those that the mussel supplies with food. The latter include such animals as birds, fishes, mollusks, crustaceans and echinoderms, which feed upon the mussels directly, and those which benefit from the metabolic activities of the mussel through its discharge of waste products and more particularly of sexual products. It was mentioned in a preceding section that a large mussel may liberate some 3 to 5 gm. (dry weight) of concentrated nutritive matter in the form of ova or spermatozoa during a single spawning season. Chemical analyses of the ripe gonads of both sexes indicate that the spermary contains 0.26% calcium and the ovary 0.16% (table 2). Since the ripe gonads consist mainly of gametes, this may be assumed to represent the percentage of that element in the gametes.

When living under favorable conditions more than 300 individuals may occupy each square meter of the surface of the rocks to which they are attached. The conclusion would follow that the population from such a minute fraction of their habitat supplies to the water no less than 1 kg. (dry weight) or about 2 pounds of gametes yearly.

Both the gametes themselves and the resulting larvae furnish a considerable part of the food supply of numerous other animal populations in the vicinity and thereby serve indirectly to replenish the nutritive elements used by the phytoplankton from which in turn the mussel derives part of its nutrition. The catabolic nitrogenous wastes of the mussel are utilized by the phytoplankton more directly.

Chemical effects. The products of respiration and excretion are used by the phytoplankton throughout the life of the mussel. After death, the calcium carbonate and other chemical elements composing the shell are slowly dissolved and after some years returned to the water from which they were taken.

The mussel is a contributor as well as a consumer of organic detritus. During life it discharges soluble nitrogenous excretory products presumably with more or less regularity and liberates at the spawning season a mass of gametes approximately equal to the weight of all its somatic tissues. At the time of death it contributes either to its predatory consumers or directly to the sea nutrients consisting of protein, lipids and carbohydrates. Most of these substances are liberated in particulate form and hence immediately available as food for the surviving members of the mussel colony or for other invertebrates, while a much smaller fraction consists of truly soluble substances which must first be consumed by bacteria or other unicellular organisms and thereby converted into particulate form before they are utilizable by multicellular animals.

SUMMARY

1. The California mussel is a mucus feeder, obtaining its food by secreting sheets of mucus over the gills; in this mucus all the microorganisms and detritus particles drawn by ciliary action into the mantle cavity are collected or adsorbed and are then carried either to the mouth or ejected from the mantle cavity as pseudofeces. By this means the mussel is able to

obtain from the water much finer particles and colloidal substances than many other filter-feeding invertebrates.

2. Growth is greatly accelerated during the third month of age, length increasing by two-thirds, and volume, tissue-weight and shell-weight four-fold. Ratios of length, volume, dry-weight of tissue and weight of shell respectively in 1-, 2- and 3-year mussels are as follows: length, 80 : 120 : 145 mm., volume, 35 : 111 : 180 cc., dry-weight of tissues, 2 : 5.6 : 8 gm., and shell-weight, 23.5 : 70 : 104.5 gm., giving linear double-logarithmic relationships between length and each of the other properties.

3. Rate of growth is dependent upon supplies of finely divided organic detritus, zoospores, floating gametes, nannoplankton and other microplankton (with special reference to dinoflagellates) utilized as food. There seems to be a generally positive correlation between water-temperature and growth rate, but with a decrease in the month of highest temperature. During 2 of the 3 years there was a generally positive correlation between growth rate of mussels and abundance of dinoflagellates.

4. Growth rate is retarded in mussels living on rocks exposed to the pounding surf and to longer periods out of water between tides. These animals possess thicker, heavier shells relative to length than the narrower, thinner-shelled phenotype found on the pilings of the pier.

5. Very young mussels of about 10 mm. length possess a slightly lower tissue-water content than the larger animals (78% vs. 83%) and nearly six-fold the concentration of tissue calcium (1.12% vs. 0.2%). Ash content is variable, with an average of about 11%. Shells are close to 93% $CaCO_3$, with small amounts of organic matter especially in the periostracum.

6. Full, ripe gonads weigh as much as or more than the somatic tissues.

7. Mussels depend upon materials present as finely divided detritus for more than four-fifths of their nutrition, living microplankton occupying a subordinate position of importance as direct food.

8. Computations show that the mussel's removal of organic matter and oxygen from the sea and its fixation of organic matter as somatic and gametic tissue are balanced by its return of gametes, CO_2 and feces to the sea. Thirty-five per cent of the intake is oxidized; 4% is fixed as gametic and somatic tissues, while the remaining 61% is unassimilated. The importance of bacteria and other microplankton in the fixing of soluble nutrients is emphasized.

9. Unfed mussels in the aquarium may lose, in 6 months time, 80% of their tissue-weight, but sustain no loss of shell substance.

10. The mussel's supply of calcium for shell-building is not available in sufficient quantity in the food but must be obtained from the water either in particulate or soluble form. Calcareous particles are ingested in large quantities. Slightly acidic conditions in the stomach and digestive diverticula would favor the dissolution of calcium salts, while the mildly alkaline nature of the mantle next the shell permits calcium deposition by that tissue.

11. Increase in size or weight was not obtained by various artificial foods nor by the addition of chalk to the water; a generous natural diet in the aquarium, with abundant aeration resulted in a moderate growth.

12. The mussel affects the physical properties of its environment by removing from the water all minute suspended objects and particles, both organic and inorganic, which enter its inhalent aperture, thus altering the turbidity and light-penetration of the water and, through the deposition of feces and pseudofeces, the character of the bottom. It alters the chemical composition of the water slightly by removing dissolved oxygen and releasing carbon dioxide and nitrogenous excretory products. By its own death or that of its gametes, it adds to the temporary supply of protein, lipid and carbohydrate

matter. In its biological effects it removes phytoplankton, including bacteria, also minute zooplankton, contributing in turn to the food supply of saprophytic and algal life by supplying catabolites, and to the nutriment of various predatory animals, including echinoderms, crustaceans, mollusks, fishes and birds. Through its gametes and free-swimming larvae it contributes directly or indirectly to the food supply of all the associated invertebrates in the vicinity and of many species of fishes.

LITERATURE CITED

BOND, RICHARD M. 1933 A contribution to the study of the natural food-cycle in aquatic environments, with particular consideration of microorganisms and dissolved organic matter. Bull. Bingham Ocean. Coll., vol. 4, art. 4, pp. 1–89.

BRANDT, K. 1898 Beiträge zur Kenntnis der chemischen Zusammensetzung des Planktons. Wiss. Meeresunters. Abt. Kiel. N. F., Bd. 3, pp. 45–90.

CLARKE, F. W., AND W. C. WHEELER 1922 The inorganic constituents of marine invertebrates. U. S. Geol. Surv., Prof. Pap., no. 124, pp. 1–22.

COE, WESLEY R., AND DENIS L. FOX 1942 Biology of the California sea mussel (Mytilus californianus). 1. Influence of temperature, food supply, sex and age on the rate of growth. J. Exp. Zool., vol. 90, pp. 1–30.

DODGSON, R. W. 1928 Report on mussel purification. Min. Agr. and Fish., Fish. Invest., vol. 10, pp. 1–498. London (H. M. Stationery Office).

FIELD, IRVING A. 1922 Biology and economic value of the sea mussel Mytilus edulis. Bull. Bureau Fish., vol. 38, pp. 127–259.

FOX, DENIS L. 1941 Changes in the tissue chloride of the California mussel in response to heterosmotic environments. Biol. Bull., vol. 80, pp. 111–129.

FOX, DENIS L., FLORENCE OLIVE AUSTIN, HORACE M. BULEY AND GRAHAM W. MARKS 1936 The habitat and food of the California sea mussel. Scripps Inst. Oceanog., Tech. Ser., vol. 4, pp. 1–64.

FOX, DENIS L., H. U. SVERDRUP AND JOHN P. CUNNINGHAM 1937 The rate of water propulsion by the California mussel. Biol. Bull., vol. 72, pp. 417–438.

GALTSOFF, PAUL S. 1934 The biochemistry of the invertebrates of the sea. Ecolog. Monogr., vol. 4, pp. 481–490.

JUDAY, C. 1942 The summer standing crop of plants and animals in four Wisconsin lakes. Trans. Wis. Acad. of Sciences, Arts and Letters, vol. 34, pp. 103–135.

KROGH, AUGUST 1934 Conditions of life in the ocean. Ecol. Monogr., vol. 4, pp. 421–429.

KROGH, A., AND A. KEYS 1934 Methods for the determination of dissolved organic carbon and nitrogen in sea water. Biol. Bull., vol. 67, pp. 132–144.

KÜHNELT, WILHELM 1938 Beziehungen zwischen Kalkstoffwechsel und Atmung bei Mollusken. Zool. Anz., vol. 124, pp. 182–190.

MacGinitie, G. E. 1941 On the method of feeding of four pelecypods. Biol. Bull., vol. 80, pp. 18–25.

Orton, J. H. 1925 The conditions for calcareous metabolism in oysters and other marine animals. Nature, vol. 116, p. 13.

Robertson, James D. 1941 The function and metabolism of calcium in the invertebrata. Biol. Rev., vol. 16, pp. 106–133.

Scheer, Bradley T. 1940 Some features of the metabolism of the carotenoid pigments in the California sea mussel (Mytilus californianus). Jour. Biol. Chem., vol. 136, pp. 275–299.

Shelford, V. E., et al. 1935 Some marine biotic communities of the Pacific coast of North America. Ecol. Monogr., vol. 5, pp. 251–354.

Whedon, W. F. 1936 Spawning habits of the mussel Mytilus californianus Conrad. Univ. Calif. Publ. Zool., vol. 41, pp. 35–44.

Whedon, W. F. and Hermann Sommer 1938 Respiratory exchange of Mytilus californianus. Zeits. vergl. Physiol., vol. 25, pp. 523–528.

White, Kathleen M. 1937 Mytilus. Liverpool Mar. Biol. Com. Memoir, vol. 31, pp. 1–117.

Yonge, C. M. 1926 Structure and physiology of the organs of feeding and digestion in Ostrea edulis. Jour. Mar. Biol. Assn., vol. 14, pp. 295–386.

Young, R. T. 1941 The distribution of the mussel (Mytilus californianus) in relation to the salinity of its environment. Ecology, vol. 22, pp. 379–386.

——— 1942 Spawning season of the California mussel. Mytilus californianus. Ecology, vol. 23, pp. 490–492.

ZoBell, C. E., and Catharine B. Feltham 1938 Bacteria as food for certain marine invertebrates. Sears Found., Jour. Marine Research, vol. 1, pp. 312–327.

ZoBell, C. E., and C. W. Grant 1942 Bacterial activity in dilute nutrient solutions. Science, vol. 96, p. 189.

——— 1943 Bacterial utilization of low concentrations of organic matter. Jour. Bact. (In press).

Contribution No. 206

STATISTICAL PROBLEMS OF STANDARDIZING THE PRECIPITATION RATIO AND ESTIMATING ITS ERROR*

By GEORGE F. MCEWEN

Scripps Institution of Oceanography†, *University of California, La Jolla, California*

The precipitation ratio, a fundamental measure introduced into climatological studies, by Dr. W. Gorczynski ("Aridity Factor and Precipitation Ratio and their relation to world climates" in this *Bulletin,* Vol. I, No. 3) is the ratio of the difference between the maximum annual precipitation and its minimum value to the mean for the number of years of record. In addition to unavoidable accidental fluctuations, the numerator of this quantity, called the range, is subject to a systematic variation with respect to the number of years of observation. Accordingly, in order to obtain comparable results, it is necessary to correct the value corresponding to each length of record used, to a constant standard length. Therefore, we shall consider certain results based on probability theory applied to the relation of range (max-min) to size of sample or length of record. A variety of results may be deduced depending upon the assumption made. Since the main purpose is to present in the allowable space, solutions obtained to date, of the various statistical problems confronting the climatologist as he works with the precipitation ratio, and since the accompanying fundamental tables are required in such practical applications, it is necessary to omit most of the details of the mathematical reasoning.

Standardization, Assuming a Normal Distribution.

First suppose that a sample of size n is drawn from a univariate normal distribution, and that the character is measured from the mean of the statistical set as origin, in terms of the standard deviation of the variate as a unit. Let u and v be respectively the largest and smallest values of the character found in the sample, so that the sample range is $w = u - v$. Then, (Pearson, 1931, p. 68) if the distribution of u in repeated samples is $y = f(u)$, the probability integral of u is

---

*Presented, in part, at the meeting of the American Mathematical Society, held at the University of California, Los Angeles, Calif., November 23, 1940.

†Contributions from the Scripps Institution of Oceanography, New Series, No. 206.

$$\int_{-\infty}^{u} f(u)\,du = \left\{ \tfrac{1}{2}\,(1 + \alpha_u) \right\}^n \qquad (1)$$

where $\frac{1}{2}(1 + \alpha_u)$ is found by entering Sheppard's tables of the normal probability function (Pearson, 1930, pp. 2-8). The expression (1) is tabulated in Pearson (1931, pp. 162-163) from which the ratio $u/\sigma = \frac{a_n}{2} = \frac{f(n)}{2}$ may be calculated for each of a series of values of n. We may also write

$$\int_{-v}^{\infty} f(u)\,du = \left\{ \tfrac{1}{2}\,(1 + \alpha_v) \right\}^n \qquad (1')$$

From symmetry the mean value of $w/\sigma = 2u/\sigma = f(n) = a_n$

Neglecting the variation of the mean with respect to n the precipitation ratio, $I_n = \frac{\text{max - min}}{\text{mean}}$ should conform to this theoretical tabulation if the distribution of the preciptation is normal. That is

$$\left[\frac{\text{max - min}}{\text{mean}}\right]_{n_1} \div \left[\frac{\text{max - min}}{\text{mean}}\right]_{n} = \frac{f(n_1)}{f(n)} = a_{n_1} \div a_n$$

where the function of (n) is tabulated in Pearson (1931, pp. 165-66) for $n = 2$ to 1000, and n is the length of the record in years. Dr. Gorczynski chose 50 as a convenient standard value of n. Accordingly the ratio $\frac{f(50)}{f(n)}$ has been tabulated (line C of Table 1) and is the factor to reduce, I_n to I_{50}, corresponding to the assumption that the variate, annual or seasonal precipitation, follows the normal law.

We may determine the median value of the greatest variate, Dodd (1923, p. 534), as follows: The probability that every one of n variates will be less than G is

$$\left[1 - \int_{G}^{\infty} \phi\,(t)\;dt\right]^n$$

where ϕ (t) is the probability function. The particular value of G such that it is equally likely that the greatest variate will or will not exceed G is called the median value. Accordingly equate this expression to ½, and obtain the following equation for the median value of the greatest variate

$$\int_{\mathrm{G} = f'(n)}^{\infty} \phi\,(t)\,dt = 1 = 1 - 2^{-1/n}$$

If ϕ is the normal probability function this equation may be reduced to the following form where G is expressed in units of σ

$$\tfrac{1}{2}\left\{1 - \frac{2}{\sqrt{2\pi}}\int_{0}^{\mathrm{G}} e^{-t2/2}\,dt\right\} = 1 - 2^{-1/n}$$

The median values of $\dfrac{\text{Range}}{\sigma}$ thus found is nearly equal to the mean value, for n not less than 5, (line C′ of table 1).

Standardization, Assuming an Asymmetrical Distribution

Second, in order to allow for asymmetry in the distribution, assume the logarithms of the variates to be distributed normally. The following notation will be used:

X = an observation, for example, the seasonal or annual precipitation.

G = the maximum value of X in a sample of n.

G' = the minimum value of X in a sample of n.

G_1 = the arithmetic mean of the value of X.

G = the geometric mean of the value of X.

$\mathrm{x} = \log \mathrm{X} = \mathrm{1n}\ \mathrm{X}$

$\mathrm{g} = \log \mathrm{G} = \mathrm{1n}\ \mathrm{G}$

$\mathrm{g}' = \log \mathrm{G}' = \mathrm{1n}\ \mathrm{G}'$

$g = \log G = \mathrm{1n}\ G$

$g_1 = \log \rho\ G_1 = \mathrm{1n}\ G_1$

Since the logarithms of X are normally distributed

$$\frac{\mathrm{G} - \mathrm{1n}\ G}{\sigma_{\mathrm{1nX}}} = \frac{\mathrm{1n}\ G - \mathrm{1n}\ \mathrm{G}'}{\sigma_{\mathrm{1nX}}} = \frac{\mathrm{g} - g}{\sigma_x} = \frac{g - \mathrm{g}'}{\sigma_x} = N = \frac{f(n)}{2} = a_{n/2}$$

also

$$G = G\,e^{N\sigma_x}$$

$$G' = G\,e^{-N\sigma_x}$$

$$\ln G - \ln G' = 2\,N\,\sigma_x$$

$$G - G' = G\,(e^{N\sigma_x} - e^{-N\sigma_x}) = 2G \sinh\,(N\,\sigma_x)$$

Therefore

$$\left[\frac{G-G'}{G}\right]_{n_I} \div \left[\frac{G-G'}{G}\right]_{n} = \frac{\sinh\left[\frac{f(n_1)}{2}\,\sigma_x\right]}{\sinh\left[\frac{f(n)}{2}\,\sigma_x\right]}$$

The precipitation ratios are

$$\left[\frac{G-G'}{G_1}\right[_{n_1} = I_{n_1}\text{, and } \left[\frac{G-G'}{G_1}\right]_n = I_n\,.$$

Therefore

$$\frac{I_{n_1}}{I_n} = \frac{\left[\frac{G-G'}{G}\right]_{n_1}\left[\frac{G}{G_1}\right]_{n_1}}{\left[\frac{G-G'}{G}\right]_{n}\left[\frac{G}{G_1}\right]_{n}} = \left(\frac{G_{n_1}\ \ G_{1_n}}{G_{1_n}\ \ G_n}\right)\frac{\sinh\left[\frac{f(n_I)}{2}\right]\sigma_x}{\sinh\left[\frac{f(n)}{2}\,\sigma_x\right]}$$

But the ratio of the geometric to arithmetic means may be regarded as independent of the length of the record or size of the sample. Therefore, the coefficient ( ) is nearly 1, and the reduction factor is, to a close approximation,

$$\frac{I_{n_1}}{I_n} = \frac{\sinh\left[\frac{f(n_I)}{2}\sigma_x\right]}{\sinh\left[\frac{f(n)}{2}\sigma_x\right]}$$

which depends upon the standard deviation, σ_x as well as upon n_I and n, lines E_1 to E_{13} of table 1. For normally distributed variates the factor depends only upon n_I and n.

Supplementary Methods of Standardization

For the third case consider the asymptotic (Dodd, 1923, p. 535) value assuming a normal distribution of the variates. The range is

$$2\,\sigma_x\sqrt{2\ \ln n} = 4.29\,\sigma_x\sqrt{\log_{10} n_1}$$

and the reduction factor is simply

$$\frac{I_{n_1}}{I_n} = \frac{\sqrt{\log_{10} n_1}}{\sqrt{\log_{10} n}}\ ,$$

line A of table 1.

For the fourth case consider the asymptotic value assuming a normal distribution of the logarithms of the variates. The formula can be derived from that applying to normally distributed variates, Dodd (1923, p. 535), by substituting logarithms of the variates thus:

$$g - g = \sigma_x\sqrt{2\ \ln n} = g - g'$$

$$g - g' = \sigma_x\sqrt{2\ \ln n}$$

$$G = G\,e^{\sigma_x\sqrt{2\ \ln n}},\quad G' = G\,e^{-\sigma_x\sqrt{2\ \ln n}},$$

$$G - G' = G\left(e^{\sigma_x\sqrt{2\ \ln n}} - e^{-\sigma_x\sqrt{2\ \ln n}}\right)$$

Therefore

$$\frac{G - G'}{G} = 2\sinh\left(\sigma_x\sqrt{2\ \ln n}\right)$$

By definition

$$I_{n_1} = \left[\frac{G - G'}{G_1}\right]_{n_1}$$

$$I_n = \left[\frac{G - G'}{G_1}\right]_n$$

Therefore

$$\frac{I_{n_1}}{I_n} = \left(\frac{G_{n_1}\ G'_n}{G1_{n_1}\ G_n}\right)\frac{\sinh\sigma_x\sqrt{2\ \ln n_1}}{\sinh\sigma_x\sqrt{2\ \ln n}}$$

Where as before the expression (   ) may be regarded as nearly equal to 1. Therefore the reduction factor is, to a close approximation,

$$\frac{I_{n_1}}{I_n} = \frac{\sinh\left[\sigma_x\sqrt{2\ \ln n_1}\right]}{\sinh\left[\sigma_x\sqrt{2\ \ln n}\right]}\ ,$$

lines D_1 to D_{10} of table 1.

For the fifth case consider the asymptotic value assuming the variates to be distributed according to the Poisson law

$$\frac{(e)^{m}\, m^{x}}{\chi\,!}$$

In this case, Dodd (1923, p. 537), $G^{G} = n$ and values of G can be calculated corresponding to a given series of values of n. Expressing the result in the form $G_n = \phi\ (n)$ we obtain the reduction formula

$$\frac{I_{n_1}}{I_n} = \frac{\phi\ (n_1)}{\phi\ (n)}\ ,$$

line B of Table 1.

Accidental Variability of the Precipitation Ratio, Normal Distribution

Computations of the precipitation ratio, I_n for a given station from successive groups of observations in a period of n years fluctuate accidentally. Accordingly, in order to obtain a measure of this variability, we shall derive formulae for computing the standard deviation, σ In. This will be done first assuming the variates (seasonal or annual precipitation) to be normally distributed and second, assuming their logarithms to be normally distributed.

W = M. R. = mean [range = max — min for an interval of n],

A = average of the n observations

σ = standard deviation of the variates.

$$\frac{\text{M. R.}}{A} = I_n,$$ precipitation ratio, by definition, and

$$\sigma_{\text{obs}} = \frac{\text{M. R.}}{f(n) = a_n},\ \sigma A = \frac{I_n A}{f(n) A} = \frac{I_n}{f(n) = a_n}$$

σ will be used for the standard deviation of the variates and is the same as $\sigma_{\text{obs.}}$

S = observed value of σ for a sample.

Consider m sets of n observations, each where N = mn = total number of observations. A series of values of W_n = (max — min), subject to chance fluctuation could be found thus for the m sets.

$\overline{W}_n$ = average of the m values of W_n.

$$W_n^{(1)} = U_n^{(1)} — \quad V_n^{(1)} \quad S_n^{(1)} \quad A_n^{(1)}$$

$$W_n^{(2)} = U_n^{(2)} — \quad V_n^{(2)} \quad S_n^{(2)} \quad A_n^{(2)}$$

. . . .

. . . .

$$W_n^{(i)} = U_n^{(i)} — \quad V_n^{(i)} \quad S_n^{(i)} \quad A_n^{(i)}$$

. . . .

. . . .

$$W_n^{(m)} = U_n^{(m)} — \quad V_n^{(m)} \quad S_n^{(m)} \quad A_n^{(m)}$$

$\sigma_{w_n} =_{b_n} \sigma$, (table 2), and $\sigma \overline{w}_n =_{b_n} \sigma / \sqrt{m}$

Likewise we could obtain a series of values of the standard deviation S of the observations by the usual formula, $S = \frac{(x — A)^2}{n — 1}$

$$\sigma_s \text{ (thus determined)} = \frac{\sigma}{\sqrt{2n}}$$

$$\sigma_{\overline{s}} = \frac{\sigma}{\sqrt{2n}\sqrt{m}}$$

where $\overline{S}$ or S = mean of the m values of S.

The last column, $A_n^{(1)}, A_n^{(2)} \ldots . A_n^{(m)}$ is the series of averages of the corresponding groups of observations, and

$A = (\Sigma_{i=1}^{i=m} A_n^{(i)})/m$

$\sigma_{A_n} = \sigma/\sqrt{n}$, and

$\sigma_A = \sigma/\sqrt{n}\sqrt{m}$

then an estimate of σ from the range would be $S = \frac{w_n}{a_n}$

$(S.\ D.)_s$, thus determined, $= \frac{1}{a_n} (S.\ D.)_{w_n}$

Therefore

$(S.\ D.)_s$, thus determined, $= (b_n/a_n)\ \sigma / \sqrt{m} = k\ (n)\ \sigma / \sqrt{2mn}$

where $k(n) = b_n \sqrt{2n} / a_n$ is a known function of (n), (see table 2). k(n) decreases from 1.511 to 1.152 for n increasing from 2 to 7 and increases from 1.152 to 1.705 for n increasing from 8 to 100

$\frac{(S.E.)_A}{(S.E.)_w} = \frac{1}{b_n \sqrt{n}}$ where values decreases from .40 to .3 to .12 as n increases from 10 to 200

$I_n = \frac{W_n}{A_n}$ where in the following the suscript will be omitted but values corresponding to n will be assumed. Therefore

$$I + \Delta I = \frac{W + \Delta W}{A + \Delta A}$$

$$\Sigma (\Delta I)^2/N_1 = [\Sigma(\Delta W)^2/N_1] I^2/W^2 + (I^2/A^2)\, \Sigma (\Delta A)^2/N_1$$

This may be reduced to

$$S_I^2 = S^2_w/A^2 + (I^2/A^2)\, S^2 A^2$$

and to

$$S_I = (I/\sqrt{2mn})\ (2\, nb_n^2/I^2 + 2)^{1/2}\, (S/A)$$

For only one group m = 1, N = n, the equation reduces to

$$S_{I_n} = (I_n/\sqrt{2n})\ (b_n \sqrt{2n}/a_n)\ \ \sqrt{1 + I_n^2/b_n^2 n}.$$

An idea regarding the value of the ratio S_{I_n}/I_n computed from this formula is given by the following condensed table

I_n	n	10	40	75
1		.28	.16	.13
2		.33	.17	.14
3		.39	.18	.15

Accidental Variability of the Precipitation Ratio, Asymmetrical Distribution

In order to allow for asymmetry, we shall compute S_I, assuming that the logs of the (obs.) are normally distributed, using the notation on p.—. Then

$$I_{\log_X} = I_x = i = \frac{2(g - g)}{g} = \frac{2(g - g')}{g}$$

$$= \frac{g - g'}{g} = \frac{\text{m. r.}}{g} = \frac{w}{g}$$

$$= \frac{\text{m. r.}}{a_n}, \; s = \text{observed value of } \sigma$$

$$\sigma_i = (i/\sqrt{2n})\;(b_n\sqrt{2n}/a_n)\sqrt{1 + i^2(^1/nb_n)^2}$$

which is not the same as the desired quantity, σ_I.

Before deriving the latter value we list tthe following approximate formulae giving relations between the standard deviation of a quantity and that of its logarithm.

$$\sigma_x^2 \sim (\sigma X/G_1^2)\{1 - \sigma_X^2/4\,G_1^2\}$$

$$\sigma_x \sim (\sigma_X/G_1)\{1 - \sigma_X^2/8\,G_1^2\} < \sigma_X/G_1$$

$$\sigma_x \sim (\sigma_X/G_1)\{1 - \sigma_x^2/8\}$$

$$\sigma_X \sim G_1\,\sigma_x\,\{1 + \sigma_x^2/8\}$$

Above assumes natural logs, or $\log_e = \ln$

For $\log_{10}$ or common logarithms

$${}_c\sigma_x^2 = (.4343)^2\;{}_n\sigma_x, {}_n\sigma_x = 2.3026\;{}_c\sigma_x$$

$${}_c\sigma_x \sim .4343\,(\sigma_X/G_1)\{1 - \sigma_X^2/8\,G_1^2\}$$

$$\sigma_X \sim G_1\,(2.3026)\;{}_c\sigma_x\{1 + (2.3026)^2\;{}_c\sigma^2{}_x/8\}$$

From the third equation above, and from certain equations of hyperboic functions, we may derive, assuming K equals $2G/G_1$ to equal 1.4, the following approximate results:

$$\sigma_I \sim \frac{G_1\,1\mathrm{n}\,(.7G_1)}{5.6}\left(1 + \frac{\sigma_i^2}{8}\right)\sigma_i,$$

$$g = 1\mathrm{n}\,(.7G_1),$$

and

$$i \sim \frac{2}{1\mathrm{n}(.7G_1)}\sinh^{-1}(1.4.\,I)$$

A more direct and accurate procedure based upon hyperbolic function theory, and the relation $I = K\sinh\left(\frac{ig}{2}\right)$ leads to the following close approximation

$$\sigma_I \sim \frac{I\,k(n)}{\sqrt{2n}} \left\{ \left(\frac{gi}{2} \right) \coth \frac{gi}{2} \right\}$$

$$\sigma_I \sim \frac{I\,k(n)}{\sqrt{2n}} \left\{ (\sinh^{-1} kI) \coth (\sinh^{-1} kI) \right\}$$

The factor { }, (see Table 4) replaces the radical, page ?? and K equals $^1/k$

Use of Several of the Greatest and of the Least Values of the Variate

The accidental error of the precipitation ratio is rather large as has been shown by dividing the variates into successive groups of constant size, n and thus obttaining a series of values of I_n for comparison. This conclusion based upon actual observation is well confirmed by theory which shows that $\frac{\sigma I_n}{I_n}$ varies from .1 to .4 for value of n from 5 to 100 and of I_n from 1.0 to 2.5. In order to reduce the effect of extreme values, we may use the average of several of the greatest and of the least values of the variate. Experience has shown that the readily obtained averages of the three highest and the three lowest variates do result in more consistent values, especially in shorter lengths of record and greater variability of precipitation. However, such values denoted by I''' are systematically smaller than those based the maximum and the minimum variates denoted by I'. Accordingly we shall attempt to derive the relation between the two and thus to compute I' from I''', in order to obtain comparable results.

First assume that the variates are distributed normally. Then the proportion of their deviations from h/σ to ∞ is

$$\frac{1}{\sqrt{2n}} \int_{h/\sigma}^{\infty} e^{-\frac{x^2}{2}}\, dx = r$$

Where $\sigma = \sigma_X =$ the standard deviation of the variates and h is the departure from the mean and depends upon σ and r. Thus assuming r, h/σ can be determined from tables of the normal probability function, and the mean, expressed in terms of σ, of the rn greatest variates is

$$a + \frac{\frac{1}{\sqrt{2n}} \int_{h/\sigma}^{\infty} x\, e^{-\frac{x^2}{2}} dx}{\frac{1}{\sqrt{2n}} \int_{h/\sigma}^{\infty} e^{-\frac{x^2}{2}} dx} = a + \frac{\frac{1}{\sqrt{2n}} e^{-\frac{h^2}{2\sigma^2}}}{r}$$

where the mean, expressed in terms of σ, is $a = \frac{G_1}{\sigma}$ Similarly, because of symmetry the mean of the rn smallest variates is

$$a + \frac{\frac{1}{\sqrt{2n}} e^{-\frac{h^2}{2\sigma^2}}}{r}$$

Therefore the mean of the rn greatest variates minus the mean of the rn smallest variates, expressed in terms of σ, is

$$\frac{2\, \frac{1}{\sqrt{2n}}\, e^{-\frac{h^2}{2\sigma^2}}}{r} = F(r) = \text{Range}_{rn}$$

If $rn = 1$, this reduces to $\text{Range}_1 = \text{maximum} - \text{minimum} = a_n$, a known function of n, expressed in terms of σ.

Therefore

$$\frac{\text{Range}_1}{\text{Range}_{rn}} = a_n \frac{r}{2 \frac{1}{\sqrt{2n}} e^{-\frac{h^2}{2\sigma^2}}} = \frac{G_b - G'_b}{G_b - G_b} =$$

$$\phi\,(n,r) = \frac{a_n}{F(r)}$$

This can be computed for any selected value of $rn = b$, which in the following development and numerical tables, was assumed to be 3. The range may also be computed from central values of the series of observations, arranged in ascending order of magnitude, by means of the following formula readily derived from the above.

$$\text{Range} = \frac{a_n R}{.7979 - (.5 - R)\, F(.5 - R)} \quad (B - B')$$

where:

B equals average of Rn terms counted from the center downward, B′ equals average of Rn terms counted from the center upward, and $(.5 - R) = r$.

In practice the assumption of a normal distribution of the variates may not be justified. Therefore, as a generalization, we shall assume the logarithms of the variates to be normally distributed. In this case we need only substitute logarithms for the variates themselves in the above formula. Therefore

$$\frac{g - g'}{g_b - g_b} = \frac{a_n}{F(r)}$$

and the problem reduces to computing $\frac{G - G'}{G_b - G'_b}$ from this equation, where $rn = b$.

Making use of the equation $G - G' = G\,2 \sinh \frac{g - g'}{2}$ from page—, we may, by using the theory of hyperbolic functions, derive the following formula

$$I'/_{I}''' = \frac{a_n}{F(r)} \sinh$$

$$\left[\frac{\sinh^{-1}\left(\frac{X_n - X_1}{2G}\right) + \sinh^{-1}\left(\frac{X_{n-1} - X_2}{2G}\right) + \sinh^{-1}\left(\frac{X_{n-2} - X_3}{2G}\right)}{3} \, \frac{a_n}{F(r)} \right]$$

$$\div \frac{\left\{ \frac{X_n + X_{n-1} + X_{n-2}}{3} - \frac{X_1 + X_2 + X_3}{3} \right\}}{2G} \, \frac{a_n}{F(r)}$$

which may be reduced to the following simpler but approximate one

$$I'/_{I}''' \sim \left(\frac{\sinh \left\{ \frac{a_n}{F(r)} \sinh^{-1}(k\,I''') + \Theta \right\}}{\frac{a_n}{F(r)} (k\,I''')} \right) \frac{a_n}{F(r)}$$

where k = (arithmetic mean ÷ 2 geometric mean)

$$= G_1 \div 2\,G \sim .7,$$

and Θ is a small quantity, plus or minus, whose value has been estimated to be usually less than 5%, in practice, and may be regarded as zero on the average. Neglecting Θ, the formula becomes

$$_{8}I \sim \left(\frac{\sinh \left[\frac{a_n}{F(r)} \sinh^{-1} \frac{G_1}{2G} I''' \right]}{\left[\frac{a_n}{F(r)} \frac{G_1}{2G} I''' \right]} \right) \frac{a_n}{F(r)} I'''$$

where $_{8}I$ is the theoretical value of I

Denote $\frac{G_1 I'''}{2G}$ by u, let $y = \sinh^{-1} u$ or $u = \sinh y$ and denote $\frac{f(n)}{F(r)}$ by $(1 + c)$. Then

$$_3I \sim \frac{\dfrac{\sinh (1 + c)\, y}{(1 + c)\, y}}{\dfrac{\sinh y}{y}} (1 + c)\, I'''$$

$$_3I \sim \left(\frac{y}{\sinh y} \quad \frac{\sinh (1 + c)\, y}{(1 + c)\, y}\right) (1 + c)\, I'''$$

The expression $\left(\frac{y}{\sinh y} \quad \frac{\sinh (1 + c)\, y}{(1 + c)\, y}\right)$ was tabulated

for $c = O$, .1, .2, .3, .4, .5,

$y =$, .5, 1.0, 1.5, 2.0, 3.0,

and the correspondent value of u:

$u =$ .521, 1.175, 2.129, 3.627, 6.050, 10.02.

Simpler approximation to this expression, within the above tabulated range, were found empirically to be $1 + .357\, uc$ and $1 + .27\, u(c + c^2)$, which is better.

In the equations

$$u = \frac{G_1 I'''}{2G}$$

a sufficiently close approximation to the geometric mean, G is the median or the square root of the product of two observations different from zero, and equi-distant from the median in the series of observations arranged in their order of magnitude. Also since $\frac{G_1}{2G}$ is usually between .5 and 1.0 we may use a constant .7 for a rough approximation to this variable ratio. Thus $u \sim .7\, I'''$ and the approximate formulae for computing I'_n from I'''_n reduce to

$$_3I_n \sim \left(1 + \left\{\left(\frac{I'''_n}{4}\right) \frac{C}{100}\right\}\right)\left[1 + \frac{C}{100}\right] I'''_n$$

The second or better one is

$$_3I'_n \sim \left\{1 + .19\, I'''_n \left[\frac{C}{100} + \left(\frac{C}{100}\right)^2\right]\right\}\left[1 + \frac{C}{100}\right] I'''_n$$

or

$$ {}_3I'_n \sim \left\{1 + .19\, I'''_n \frac{C}{100}\left[1 + \frac{C}{100}\right]\right\}\left[1 + \frac{C}{100}\right] I'''_n, $$

where C = the tabular entry, expressed in per cent (Table 5).

In about 200 series of precipitation observations representing a wide range of conditions both I'_n and I'''_n were determined directly, and the formulae were used to compute I'_n from I'''_n. In general a very good agreement resulted from the simpler rule ${}_3I_n \sim$

$\left[1 + \frac{C}{100}\right] I'''_n$ if $I'''_n < 2.8$. For $I_n > 2.8$ the factor

$\{\quad\}$, in the first formula, should be included.

For convenience in applying the formulae, C and the function,

$f(C) = .19C\left[1 + \frac{C}{100}\right]^2$ are presented in Table 5. The

second formula may be reduced to the form

$$ {}_3I'_n = I'''_n + \frac{C}{100}\, I'''_n + \frac{f(C)}{100}\, (I'''_n)^2 $$

which can be readily used with the aid of Table 5. The value, ${}_3I_n$ thus computed is subject to en error Θ, page —, which will usually not exceed 5 per cent of ${}_3I_n$.

Graphical Standardization of the Precipitation Ratio by Means of Log-Reciprocal Coordinate Paper.

Let I_{50} be the repiciptation ratio corresponding to 50 years and I_n be that for n years. It was found by trial that the ratios I_{50}/I_n and values of n would plot as approximately straight lines on paper with a logarithmic scale for n and a reciprocal scale for I_{50}/I_n, Figure. 1. Since the ratio I_{50}/I_n multiplied by I_n gives the standardized value I_{50} corresponding to 50 years, an appropriate name would be reduction factor.

Such a plotting chart (Fig. 1) serves to adjust a series of observed values of I_{50}/I_n for various values of n, and to determine

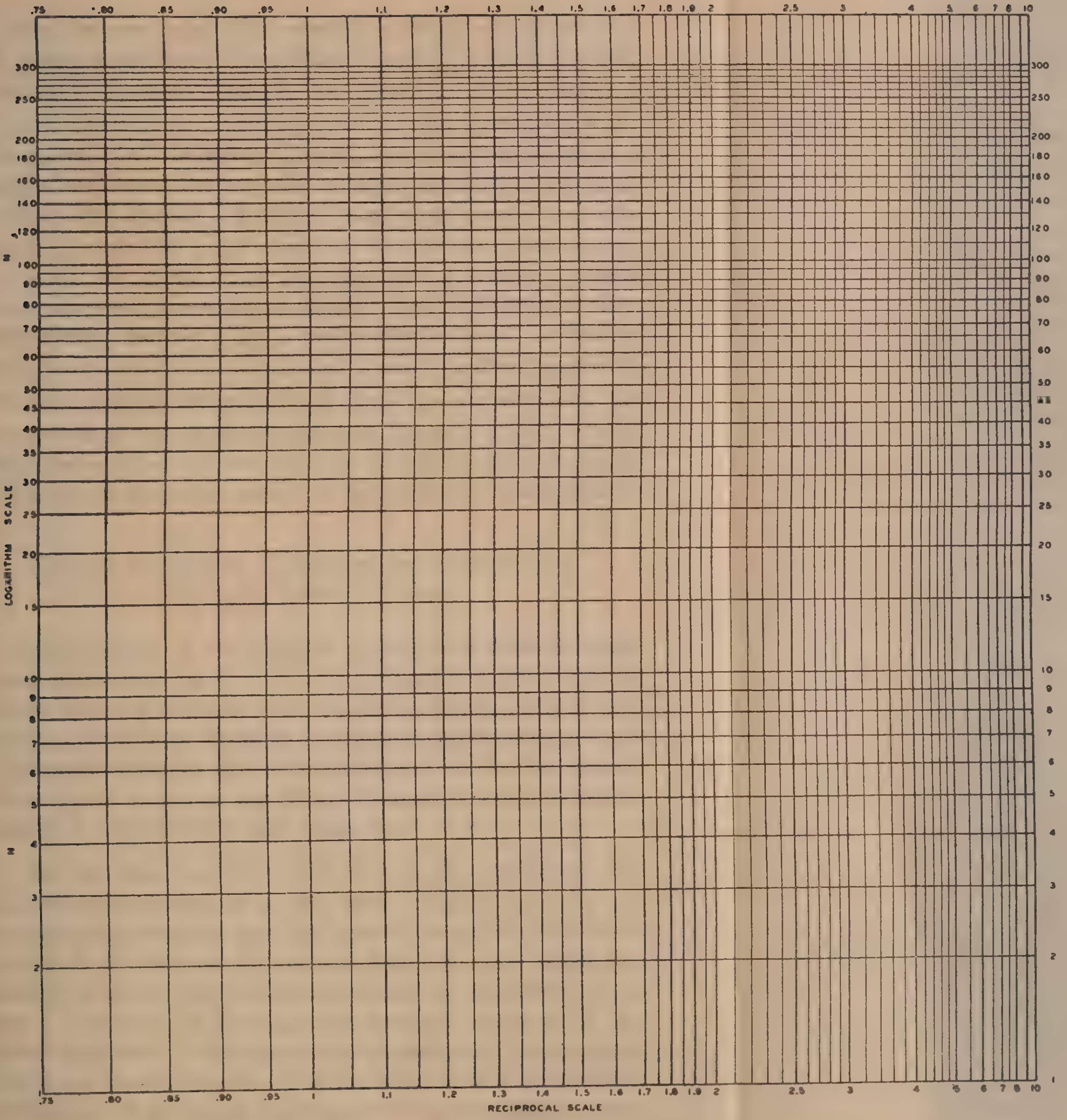


FIG. 1

Chart for Plotting Rations $\lambda\ In_1/I_n$ and Determining the Reduction Factor. For Standardizing the Precipitation Ratio.

I_{50} from the same series. First estimate or assume a trial value of the unknown standardized precipitation ratio I_{n_1} Denote this trial value by $\lambda\ I_{n_1}$ where λ and I_{n_1} are unknown, plot the ratios $\lambda\ I_{n_1}/I_n$ on the reciprocal scale against n on the logarithmic scale. The smooth curve determined by these points will approximate a straight line and can be projected to obtain extrapolated values of the ratios. The abscissa, $\underset{\lambda}{\lambda}\ I_{n_1}/I_n$ of a point on the curve whose ordinate is n_1, is λ which would equal 1 if the trial value were correct. If λ is not one, divide all of the ratios by λ, plot these corrected values which will determine an adjusted curve that will pass more nearly through the point $(1, n_1)$. Any assumed value $\lambda\ I_{n_1}$ divided by λ will give the standardized precipitation ratio I_{n_1} and the graphically smoothed values of the ratios I_{n_1}/I_n, based upon observations, can be regarded as the empirical reduction factors for that locality. These empirical or observed reduction factors can then be readily compared with theoretical ones as is done in Figure 2 and Table 6.

Types of Precipitation Distribution, and Corresponding Theoretical Reduction Factors

An examination of this material together with other examples clearly shows that one of a few selected series of theoretical reduction factors agrees well with the factors for any type of precipitation distribution. Moreover as table 9 shows, the average value of factors adopted for use in the earlier part of Dr. Gorczynski's climatological studies at the Scripps Institution are approximate medians of the theoretical ones found later.

It has not been possible to formulate a very satisfactory rule for selecting the proper set of theoretical reduction factors to use in a given case. However there is a general progression from series A to E_7 as the value of I_{50} increases from 1.2 to 4.0 which corresponds to extreme desert conditions. Using the approximate rule, page —, that $.21\ I_{50} = \sigma_{\mathrm{InX}}$ = the standard deviation of the natural logarithms of the precipitation we may make the following comparison remembering that .1 of the subscript of E is the standard deviation of the natural logarithms of the precipitation assumed in computing the theoretical factors.

Tabulation of .2 I_{50} for various groups of observations in columns designating the corresponding theoretical series

	A	C, E_I	E_3	E_4,E_5	E_7
	.26	.28	.08	.28	.15
	(.28)	.30	.09	.28	.20
	.42	(.30)	.11	.44	.20
		.30	.13	(.44)	.30
		.49	(.20)	(.50)	.32
			.28	.56	.43
			.28	.68	.49
			.30	.82	.58
			.44		(.58)
					.60
					.64
					.80
					1.04
					1.4
					1.4
					1.4
Average					
	.32	.334	.212	.50	.66

This table shows that the median or average values of .2 1_{50} are roughly equal to the expected ones; that is, to .1 of the subscript of E. It is also evident that this positive correlation is especially good for the last three groups which represent a progressive approach to extreme desert conditions.

After this paper was written my attention was called to one by Simpson (1941) dealing with a similar problem in the field of zoology. He emphasizes the need of standardization to a constant number of observations and considers the reliability of the range, assuming a normal distribution of the variates.

FACTORS PLOTTED ON RECIPROCAL AND LOGARITHMIC SCALES FOR STANDARDIZING THE PRECIPITATION RATIO TO A FIFTY-YEAR INTERVAL

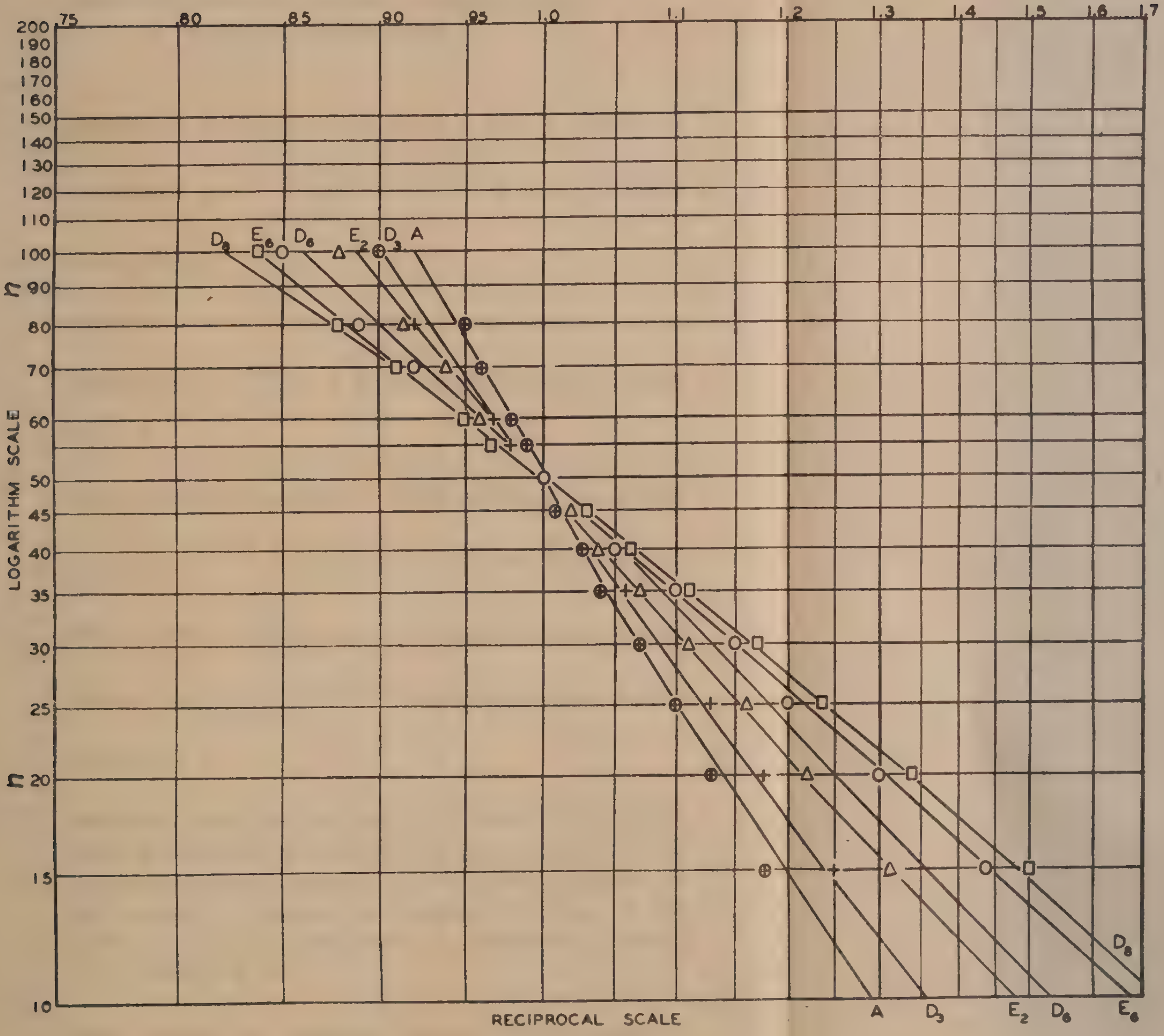


FIG. 2

Curves show theoretical factors plotted from Table I.

The letters indicate theoretical factors tabulated in Table I. that agree approximately with the observations.

Points correspond to factors computed from various typical groups of observations, listed on the opposite page.

TABLE I.

EXPLANATION OF SYMBOLS INDICATING BASIC ASSUMPTIONS.

Symbol	
A	Asymptotic value of the range of a variate, subject to the normal law $\frac{1}{\sigma\sqrt{2n}} e^{-x^2/2\sigma^2}$ is $2\sigma\sqrt{2\,\mathrm{In}^{n}} = 4.29\sigma\sqrt{\log_{10}{}^{n}}$.
B	Asymptotic value of the greatest variate, subject to the Poisson law $\frac{e^{\,m}m^{x}}{x}$, is X computed from the equation $X^{X} = n$.
C	Mean value of the range of a variate, subject to the normal $\frac{1}{\sigma\sqrt{2n}} e^{-x^2/2\sigma^2}$ is $a_n\sigma$ where a_n is a known function of n.
C′	Median value of the range of a variate, subject to the normal law $\frac{1}{\sigma\sqrt{2n}} e^{-x^2/2\sigma^2}$ is $a'_n\sigma$ where a'_n is a known function of n.
D	Asymptotic value of the range of a variate, whose logarithm is subject to the normal law, is sinh $[\sigma_{1n}\sqrt{2\,1n\,n}]$ equals the standard deviation of the natural logarithm of the variate. σ_{In} equals the standard deviation of the natural logarithm of the variate. σ_{In}, expressed in tenths, is the subject of D.
E	Mean value of the range of a variate whose logarithm is subject to the normal law, is sinh $\left[\frac{a_n\sigma_{In}}{2}\right]$ where σ_{1n}, expressed in tenths, is the subscript of E.

TABLE 1

THEORETICAL VALUES OF FACTORS REQUIRED TO REDUCE THE RANGE CORRESPONDING TO A GROUP OF n VARIATES TO A GROUP OF 50 VARIATES SELECTED AS A STANDARD.

n =	5	10	15	20	25	30	35	40	60	70	80	100
Symbol Indicating Basic Assumptions												
A	1.558	1.303	1.202	1.142	1.095	1.073	1.048	1.029	0.977	0.960	0.944	0.921
B	1.543	1.312	1.212	1.152	1.109	1.077	1.052	1.032	0.975	0.956	0.940	0.914
C	1.932	1.462	1.295	1.204	1.144	1.101	1.067	1.040	0.970	0.946	0.927	0.897
C′	1.952	1.470	1.301	1.208	1.147	1.103	1.069	1.041	0.969	0.945	0.925	0.895
D_1	1.578	1.315	1.209	1.150	1.105	1.076	1.052	1.033	0.979	0.963	0.947	0.922
D_2	1.609	1.333	1.222	1.157	1.113	1.081	1.054	1.033	0.975	0.956	0.939	0.913
D_3	1.668	1.368	1.245	1.173	1.124	1.089	1.060	1.036	0.973	0.951	0.933	0.903
D_4	1.753	1.414	1.276	1.196	1.141	1.100	1.068	1.042	0.970	0.943	0.923	0.891
D_5	1.862	1.474	1.316	1.224	1.161	1.114	1.078	1.047	0.964	0.936	0.913	0.876
D_6	1.994	1.545	1.363	1.256	1.184	1.130	1.088	1.054	0.960	0.927	0.900	0.859
D_7	2.155	1.628	1.417	1.294	1.211	1.148	1.101	1.061	0.954	0.918	0.888	0.842

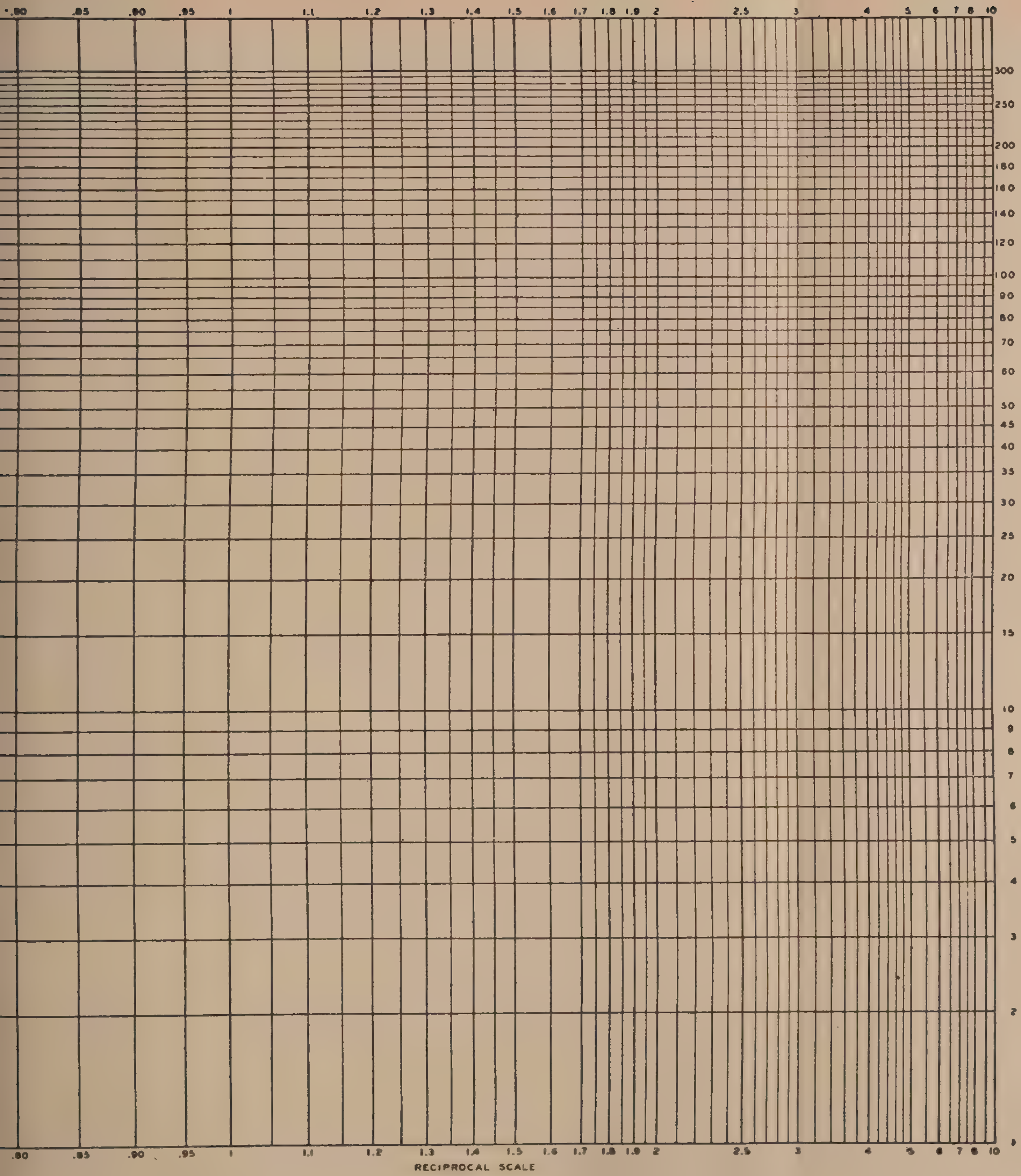


FIG. 1

r Plotting Rations λ In_1/I_n and Determining the Reduction Factor. For Standardizing the Precipitation Ratio.

I_{50} from the same series. First estimate or assume a trial value of the unknown standardized precipitation ratio I_{n_1} Denote this trial value by $\lambda\ I_{n_1}$ where λ and I_{n_1} are unknown, plot the ratios $\lambda\ I_{n_1}/I_n$ on the reciprocal scale against n on the logarithmic scale. The smooth curve determined by these points will approximate a straight line and can be projected to obtain extrapolated values of the ratios. The abscissa, $\underset{\lambda}{\lambda}\ I_{n_1}/I_n$ of a point on the curve whose ordinate is n_1, is λ which would equal 1 if the trial value were correct. If λ is not one, divide all of the ratios by λ, plot these corrected values which will determine an adjusted curve that will pass more nearly through the point $(1, n_1)$. Any assumed value $\lambda\ I_{n_1}$ divided by λ will give the standardized precipitation ratio I_{n_1} and the graphically smoothed values of the ratios I_{n_1}/I_n, based upon observations, can be regarded as the empirical reduction factors for that locality. These empirical or observed reduction factors can then be readily compared with theoretical ones as is done in Figure 2 and Table 6.

Types of Precipitation Distribution, and Corresponding Theoretical Reduction Factors

An examination of this material together with other examples clearly shows that one of a few selected series of theoretical reduction factors agrees well with the factors for any type of precipitation distribution. Moreover as table 9 shows, the average value of factors adopted for use in the earlier part of Dr. Gorczynski's climatological studies at the Scripps Institution are approximate medians of the theoretical ones found later.

It has not been possible to formulate a very satisfactory rule for selecting the proper set of theoretical reduction factors to use in a given case. However there is a general progression from series A to E_7 as the value of I_{50} increases from 1.2 to 4.0 which corresponds to extreme desert conditions. Using the approximate rule, page —, that $.21\ I_{50} = \sigma_{\ln x}$ = the standard deviation of the natural logarithms of the precipitation we may make the following comparison remembering that .1 of the subscript of E is the standard deviation of the natural logarithms of the precipitation assumed in computing the theoretical factors.

Tabulation of .2 I_{50} for various groups of observations in columns designating the corresponding theoretical series

A	C, E_1	E_3	E_4,E_5	E_7
.26	.28	.08	.28	.15
(.28)	.30	.09	.28	.20
.42	(.30)	.11	.44	.20
	.30	.13	(.44)	.30
	.49	(.20)	(.50)	.32
		.28	.56	.43
		.28	.68	.49
		.30	.82	.58
		.44		(.58)
				.60
				.64
				.80
				1.04
				1.4
				1.4
				1.4
Average				
.32	.334	.212	.50	.66

This table shows that the median or average values of .2 1_{50} are roughly equal to the expected ones; that is, to .1 of the subscript of E. It is also evident that this positive correlation is especially good for the last three groups which represent a progressive approach to extreme desert conditions.

After this paper was written my attention was called to one by Simpson (1941) dealing with a similar problem in the field of zoology. He emphasizes the need of standardization to a constant number of observations and considers the reliability of the range, assuming a normal distribution of the variates.

FACTORS PLOTTED ON RECIPROCAL AND LOGARITHMIC SCALES FOR STANDARDIZING THE PRECIPITATION RATIO TO A FIFTY-YEAR INTERVAL

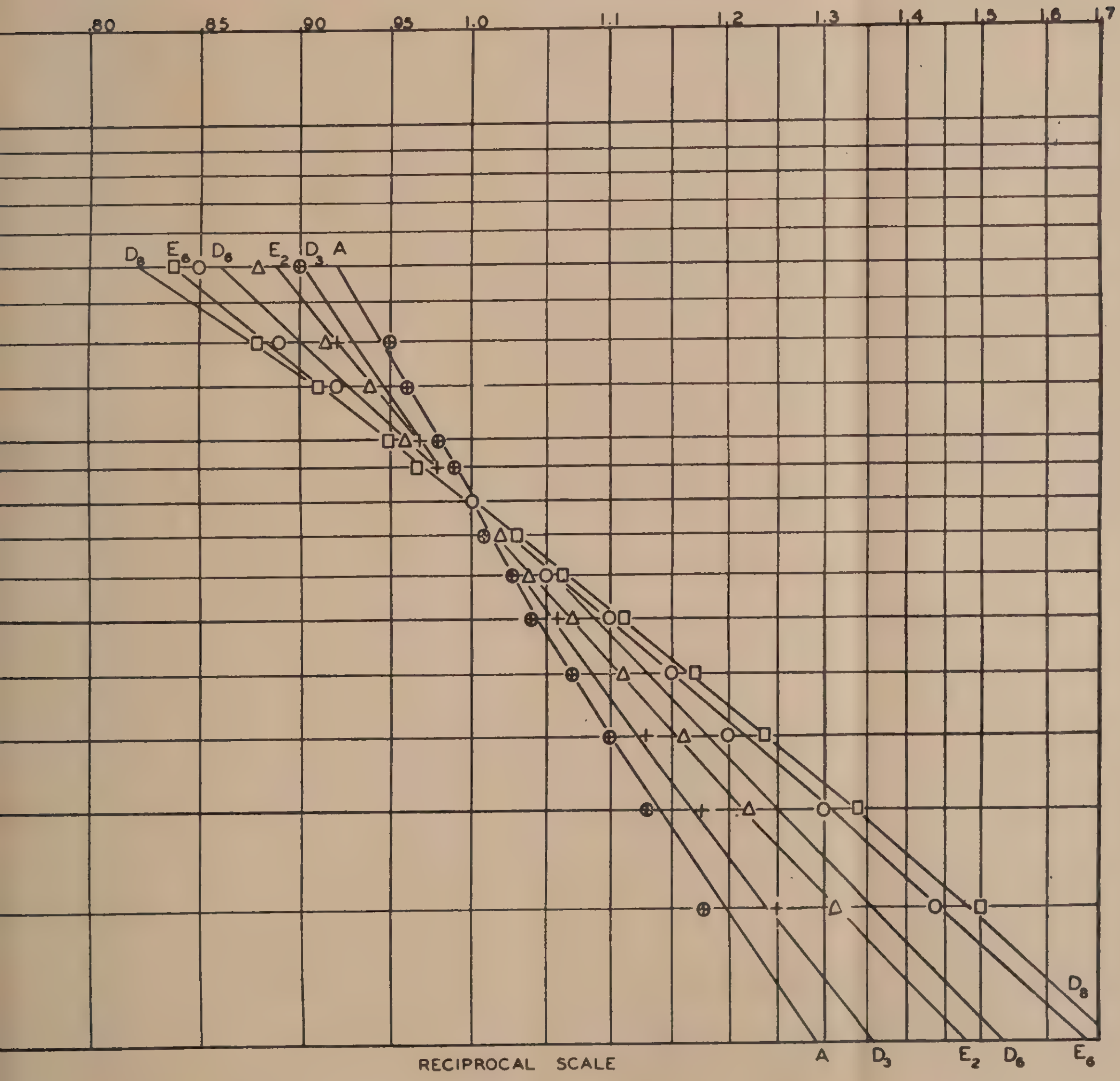


FIG. 2

Curves show theoretical factors plotted from Table I.

letters indicate theoretical factors tabulated in Table I. that agree approximately with the observations.

correspond to factors computed from various typical groups of observations, listed on the opposite page.

TABLE I.

EXPLANATION OF SYMBOLS INDICATING BASIC ASSUMPTIONS.

Symbol	
A	Asymptotic value of the range of a variate, subject to the normal law $\frac{1}{\sigma\sqrt{2n}}\, e^{-x^2/2\sigma^2}$ is $2\,\sigma\,\sqrt{2\,\mathrm{In}^{n}} =$ $4.29\sigma\sqrt{\log_{10}{}^{n}}$.
B	Asymptotic value of the greatest variate, subject to the Poisson law $\frac{e^{m}m^{x}}{x}$, is X computed from the equation $X^{X} = n$.
C	Mean value of the range of a variate, subject to the normal $\frac{1}{\sigma\sqrt{2n}}\, e^{-x^2/2\sigma^2}$ is $a_n\sigma$ where a_n is a known function of n.
C′	Median value of the range of a variate, subject to the normal law $\frac{1}{\sigma\sqrt{2n}}\, e^{-x^2/2\sigma^2}$ is $a'_n\sigma$ where a'_n is a known function of n.
D	Asymptotic value of the range of a variate, whose logarithm is subject to the normal law, is sinh $\left[\sigma_{1n}\sqrt{2\ 1n\ n}\right]$ equals the standard deviation of the natural logarithm of the variate. σ_{In} equals the standard deviation of the natural logarithm of the variate. σ_{In}, expressed in tenths, is the subject of D.
E	Mean value of the range of a variate whose logarithm is subject to the normal law, is sinh $\left[\frac{a_n\sigma_{In}}{2}\right]$ where σ_{1n}, expressed in tenths, is the subscript of E.

TABLE 1

THEORETICAL VALUES OF FACTORS REQUIRED TO REDUCE THE RANGE CORRESPONDING TO A GROUP OF n VARIATES TO A GROUP OF 50 VARIATES SELECTED AS A STANDARD.

n =	5	10	15	20	25	30	35	40	60	70	80	100
Symbol Indicating Basic Assumptions												
A	1.558	1.303	1.202	1.142	1.095	1.073	1.048	1.029	0.977	0.960	0.944	0.921
B	1.543	1.312	1.212	1.152	1.109	1.077	1.052	1.032	0.975	0.956	0.940	0.914
C	1.932	1.462	1.295	1.204	1.144	1.101	1.067	1.040	0.970	0.946	0.927	0.897
C′	1.952	1.470	1.301	1.208	1.147	1.103	1.069	1.041	0.969	0.945	0.925	0.895
D_1	1.578	1.315	1.209	1.150	1.105	1.076	1.052	1.033	0.979	0.963	0.947	0.922
D_2	1.609	1.333	1.222	1.157	1.113	1.081	1.054	1.033	0.975	0.956	0.939	0.913
D_3	1.668	1.368	1.245	1.173	1.124	1.089	1.060	1.036	0.973	0.951	0.933	0.903
D_4	1.753	1.414	1.276	1.196	1.141	1.100	1.068	1.042	0.970	0.943	0.923	0.891
D_5	1.862	1.474	1.316	1.224	1.161	1.114	1.078	1.047	0.964	0.936	0.913	0.876
D_6	1.994	1.545	1.363	1.256	1.184	1.130	1.088	1.054	0.960	0.927	0.900	0.859
D_7	2.155	1.628	1.417	1.294	1.211	1.148	1.101	1.061	0.954	0.918	0.888	0.842

D_8	2.341	1.722	1.476	1.334	1.239	1.168	1.114	1.069	0.949	0.908	0.875	0.824
D_9	2.553	1.824	1.540	1.377	1.269	1.188	1.127	1.077	0.943	0.898	0.861	0.805
D_{10}	2.797	1.939	1.609	1.423	1.300	1.210	1.141	1.085	0.938	0.888	0.848	0.787
E_1	1.957	1.474	1.305	1.207	1.152	1.107	1.071	1.041	0.970	0.946	0.927	0.897
E_2	1.979	1.490	1.314	1.217	1.154	1.110	1.071	1.043	0.969	0.943	0.923	0.891
E_3	2.042	1.521	1.334	1.232	1.165	1.115	1.077	1.048	0.965	0.938	0.916	0.881
E_4	2.129	1.566	1.366	1.254	1.181	1.126	1.086	1.051	0.962	0.932	0.909	0.869
E_5	2.239	1.626	1.404	1.281	1.199	1.140	1.094	1.057	0.958	0.925	0.898	0.856
E_6	2.377	1.695	1.448	1.313	1.221	1.156	1.104	1.063	0.953	0.916	0.886	0.840
E_7	2.546	1.779	1.502	1.349	1.247	1.174	1.116	1.070	0.948	0.907	0.875	0.823
E_8	2.747	1.879	1.562	1.390	1.276	1.193	1.129	1.078	0.943	0.898	0.862	0.806
E_9	2.977	1.988	1.631	1.436	1.307	1.214	1.144	1.086	0.937	0.888	0.849	0.788
E_{10}	3.247	2.111	1.705	1.484	1.340	1.237	1.158	1.095	0.931	0.878	0.835	0.769
E_{11}	3.556	2.247	1.785	1.537	1.375	1.261	1.173	1.105	0.925	0.867	0.821	0.751
E_{12}	3.904	2.397	1.872	1.593	1.412	1.286	1.190	1.114	0.919	0.857	0.808	0.738
E_{13}	4.302	2.561	1.964	1.652	1.452	1.312	1.206	1.124	0.913	0.846	0.794	0.714

TABLE 2

TABLES OF VARIOUS FUNCTIONS OF THE LENGTH OF INTERVAL, n FOR COMPUTING THE STANDARD DEVIATION OF THE PRECIPITATION RATIO, I_n.

EXPLANATION

Assume a normal distribution of the observations where u = the maximum and v = the minimum in a sample of size n. σ = standard deviation of the observations. u — v = range or interval of variation = $a_n\sigma$.
Standard deviation of the range $= b_n\sigma$ Standard deviation of the standard deviation (determined by the range) =

$$\frac{b_n}{a_n}\ \sigma = k(n)\ \frac{\sigma}{\sqrt{2n}}$$

n	$\sqrt{2n}$	a_n	b_n	$k(n)$	$\frac{1}{nb_n^2}$	$\frac{k(n)}{\sqrt{2n}} = \frac{b_n}{a_n}$	F(r)	$\frac{a_n}{F(r)}$
5	3.162	2.326	.8641	1.175	.2678	.3715		
6	3.464	2.534	.8480	1.160	.2314	.3346	1.5957	1.5883
7	3.742	2.704	.8330	1.164	.2052	.3080	1.8315	1.4766
8	4.000	2.847	.8200	1.153	.1858	.2880	2.0222	1.4079
9	4.243	2.970	.8080	1.154	.1706	.2721	2.1810	1.3617
10	4.472	3.077	.7970	1.158	.1576	.2590	2.3180	1.3276
11	4.690	3.173	.7870	1.162	.1468	.2480	2.4372	1.3018
12	4.899	3.258	.7780	1.168	.1377	.2388	2.5426	1.2816
13	5.099	3.360	.7700	1.175	.1301	.2308	2.6357	1.2657
14	5.292	3.407	.7620	1.183	.1230	.2237	2.7211	1.2520
15	5.477	3.472	.7550	1.192	.1170	.2175	2.7995	1.2410
16	5.657	3.532	.7490	1.200	.1113	.2121		

17	5.831	3.588	.7430	1.208	.1062	.2071		
18	6.000	3.640	.7380	1.217	.1017	.2027		
19	6.164	3.689	.7330	1.225	.0977	.1987		
20	6.325	3.735	.7290	1.234	.0942	.1952	3.1085	1.2015
21	6.481	3.778	.7250	1.243	.0907	.1919		
22	6.633	3.819	.7200	1.252	.0875	.1885		
23	6.782	3.858	.7160	1.261	.0847	.1856		
24	6.928	3.895	.7130	1.270	.0820	.1831		
25	7.071	3.931	.7090	1.278	.0795	.1804	3.3344	1.1788
26	7.211	3.964	.7050	1.286	.0773	.1779		
27	7.348	3.996	.7020	1.293	.0751	.1757		
28	7.483	4.027	.6990	1.300	.0731	.1736		
29	7.616	4.057	.6960	1.307	.0712	.1716		
20	6.325	3.735	.7290	1.234	.0942	.1952	3.1085	1.2015
25	7.071	3.931	.7090	1.278	.0795	.1804	3.3344	1.1788
30	7.746	4.085	.6930	1.314	.0694	.1696	3.5100	1.1640
35	8.367	4.213	.6810	1.350	.0615	.1616	3.6550	1.1572
40	8.944	4.321	.6700	1.387	.0557	.1551	3.7750	1.1448
45	9.487	4.415	.6600	1.418	.0511	.1495	3.8775	1.1387
50	10.000	4.498	.6520	1.450	.0470	.1449	3.9714	1.1331
55	10.488	4.572	.6450	1.480	.0437	.1411	4.0519	1.1284
60	10.954	4.639	.6390	1.510	.0408	.1378	4.1254	1.1244
65	11.402	4.699	.6330	1.536	.0384	.1347	4.1929	1.1212
70	11.832	4.755	.6280	1.560	.0361	.1321	4.2517	1.1183
75	12.247	4.806	.6240	1.589	.0342	.1298	4.3085	1.1155
80	12.649	4.854	.6190	1.614	.0328	.1275	4.3611	1.1129
85	13.038	4.898	.6150	1.638	.0311	.1256	4.4072	1.1113
90	13.416	4.939	.6120	1.663	.0296	.1239	4.4543	1.1089
95	13.784	4.978	.6080	1.684	.0285	.1221	4.4944	1.1077
100	14.142	5.015	.6050	1.707	.0273	.1206	4.5351	1.1056

EMPIRICAL FACTORS TO REDUCE THE PRECIPITATION RATIO I_n, BASED ON n YEARS TO THAT FOR 50 YEARS AND COMPUTED FROM THE OBSERVATIONS

REGION	I_{50} n =	15	20	25	30	35	40	45	50	55	60	70	80	100	
Southern California															
⊕ El Cajon, Escondido, Poway	1.2-1.4	1.18	1.13	1.10	1.07	1.04	1.03	1.01	1.00	.99	.98	.96	.95	.90	A
+ Santa Monica, San Diego Bonita, Chula Vista	1.3-1.6	1.25	1.18	1.13	1.10	1.06	1.03	1.02	1.00	.98	.97	.94	.92	.88	D_3
△ Bagdad, Barstow, Blythe Indio, Parker	1.9-5.0	1.31	1.22	1.16	1.11	1.07	1.04	1.02	1.00	.97	.96	.94	.91	.88	E_2
● Pacific Islands	.8-2.4	1.44	1.30	1.20	1.15	1.10	1.05	1.02	1.00	.97	.95	.92	.89	.85	E_6
☐ Arabian Desert Region	2.0-4.0	1.50	1.34	1.24	1.17	1.11	1.06	1.03	1.00	.97	.95	.91	.88	.84	D_8

TABLE 3

FACTORS IN PER CENT, FOR REDUCING THE PRECIPITATION RATIO CORRESPONDING TO n YEARS TO THAT FOR A 50 YEAR PERIOD

(Empirical values based upon observations and used in the early part of the work)

n Years	0	1	2	3	4	5	6	7	8	9
0						109	92	78	69	60
10	55	50	46	42	40	36	33	30	28	26
20	24	23	22	21	20	19	18	16	15	14
30	13	12	11	10	9	9	8	8	7	7
40	6	5	5	4	3	3	2	2	1	0
50	0	0	—1	—2	—2	—2	—2	—2	—3	—3
60	—3	—3	—4	—5	—5	—5	—5	—6	—6	—6
70	—6	—7	—7	—7	—7	—7	—7	—8	—8	—8
80	—8	—9	—9	—9	—9	—10	—10	—10	—10	—11
90	—11	—11	—12	—12	—12	—12	—12	—12	—13	—13
100	—13	—13	—13	—14	—14	—14	—14	—14	—14	—14

TABLE 4

TABULATION OF Z (coth Z) = Z/ (tanh Z).

Z	.00	.01	.02	.03	.04	.05	.06	.07	.08	.09
0	1.0	1.0000	1.0000	1.0003	1.0005	1.0008	1.0012	1.0016	1.0021	1.0027
.1	1.0033	1.0040	1.0048	1.0056	1.0065	1.0075	1.0085	1.0096	1.0108	1.0120
.2	1.0133	1.0146	1.0161	1.0176	1.0191	1.0207	1.0224	1.0242	1.0260	1.0279
.3	1.0298	1.0318	1.0339	1.0360	1.0382	1.0405	1.0428	1.0452	1.0477	1.0502
.4	1.0528	1.0554	1.0581	1.0609	1.0637	1.0666	1.0696	1.0726	1.0757	1.0788
.5	1.0820	1.0852	1.0885	1.0919	1.0954	1.0989	1.1024	1.1060	1.1097	1.1134
.6	1.1172	1.1211	1.1250	1.1289	1.1329	1.1370	1.1412	1.1453	1.1496	1.1539
.7	1.1582	1.1626	1.1671	1.1716	1.1762	1.1808	1.1855	1.1902	1.1950	1.1999
.8	1.2047	1.2097	1.2147	1.2197	1.2248	1.2300	1.2352	1.2404	1.2457	1.2511
.9	1.2565	1.2619	1.2674	1.2729	1.2785	1.2842	1.2898	1.2956	1.3013	1.3072
1.0	1.3130	1.3190	1.3249	1.3309	1.3369	1.3430	1.3492	1.3554	1.3616	1.3678
1.1	1.3741	1.3805	1.3869	1.3933	1.3998	1.4063	1.4128	1.4194	1.4261	1.4327
1.2	1.4395	1.4462	1.4530	1.4598	1.4667	1.4736	1.4805	1.4875	1.4945	1.5015
1.3	1.5086	1.5157	1.5229	1.5301	1.5373	1.5445	1.5518	1.5591	1.5665	1.5739
1.4	1.5813	1.5888	1.5962	1.6037	1.6113	1.6189	1.6265	1.6341	1.6418	1.6495

1.5	1.6572	1.6649	1.6727	1.6805	1.6884	1.6962	1.7041	1.7120	1.7200	1.7280
1.6	1.7360	1.7440	1.7521	1.7601	1.7683	1.7764	1.7845	1.7927	1.8009	1.8091
1.7	1.8174	1.8257	1.8340	1.8423	1.8506	1.8590	1.8674	1.8758	1.8842	1.8927
1.8	1.9011	1.9096	1.9181	1.9267	1.9352	1.9438	1.9524	1.9610	1.9696	1.9783
1.9	1.9869	1.9956	2.0043	2.0131	2.0218	2.0306	2.0394	2.0481	2.0570	2.0658
2.0	2.0746	2.0835	2.0924	2.1013	2.1102	2.1191	2.1280	2.1370	2.1460	2.1550
2.1	2.1639	2.1730	2.1820	2.1910	2.2001	2.2091	2.2182	2.2273	2.2364	2.2456
2.2	2.2547	2.2638	2.2730	2.2822	2.2913	2.3005	2.3098	2.3190	2.3282	2.3375
2.3	2.3467	2.3560	2.3652	2.3745	2.3838	2.3931	2.4025	2.4118	2.4211	2.4305
2.4	2.4398	2.4492	2.4586	2.4680	2.4774	2.4868	2.4962	2.5056	2.5150	2.5245
2.5	2.5339	2.5434	2.5528	2.5623	2.5718	2.5813	2.5908	2.6003	2.6098	2.6193
2.6	2.6288	2.6384	2.6479	2.6575	2.6670	2.6766	2.6862	2.6957	2.7053	2.7149
2.7	2.7245	2.7341	2.7437	2.7533	2.7629	2.7726	2.7822	2.7918	2.8015	2.8111
2.8	2.8208	2.8304	2.8401	2.8498	2.8594	2.8691	2.8788	2.8885	2.8982	2.9079
2.9	2.9176	2.9273	2.9370	2.9468	2.9565	2.9662	2.9760	2.9857	2.9954	3.0051
3.0	3.0149	3.0247	3.0344	3.0442	3.0540	3.0637	3.0735	3.0833	3.0931	3.1028

TABLE 5

TABULATION OF CORRECTION FACTORS FOR REDUCING I'''_n to I'_n

First line, C, and Second line, $f(C) = .19C \left(1 + \frac{100}{C}\right)^2$, both in percentage.

n yrs	0	1	2	3	4	5	6	7	8	9
0	—	—	—	—	—	—	58.8	47.7	40.8	36.2
							28.2	19.9	15.4	13.0
10	32.8	30.2	28.2	26.6	25.2	24.0	23.0	22.2	21.4	20.7
	11.0	9.7	8.8	8.1	7.5	7.0	6.6	6.3	6.0	5.7
20	20.1	19.6	19.1	18.7	18.3	17.9	17.5	17.2	17.0	16.7
	5.5	5.3	5.1	5.0	4.9	4.7	4.6	4.5	4.4	4.3
30	16.4	16.1	16.0	15.7	15.5	15.3	15.1	15.0	14.8	14.6
	4.2	4.1	4.1	4.0	3.9	3.9	3.8	3.8	3.7	3.6
40	14.4	14.3	14.2	14.0	13.9	13.9	13.7	13.6	13.5	13.4
	3.6	3.5	3.5	3.5	3.4	3.4	3.4	3.3	3.3	3.3
50	13.3	13.2	13.1	13.0	12.9	12.8	12.8	12.7	12.6	12.5
	3.2	3.2	3.2	3.2	3.1	3.1	3.1	3.1	3.0	3.0
60	12.5	12.4	12.3	12.2	12.2	12.1	12.1	12.0	11.9	11.9
	3.0	3.0	2.9	2.9	2.9	2.9	2.9	2.9	2.8	2.8
70	11.8	11.7	11.7	11.6	11.6	11.5	11.4	11.4	11.3	11.3
	2.8	2.8	2.8	2.7	2.7	2.7	2.7	2.7	2.7	2.7
80	11.2	11.2	11.2	11.1	11.1	11.0	11.0	11.0	10.9	10.9
	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.5	2.5
90	10.9	10.9	10.8	10.8	10.8	10.8	10.7	10.7	10.7	10.7
	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
100	10.6	10.6	10.5	10.5	10.5	10.4	10.4	10.3	10.3	10.2
	2.5	2.5	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4

TABLE 5

TABULATION OF CORRECTION FACTORS FOR REDUCING I'''_n to I'_n

First line C, and Second line, $f(C) = .19C \left(1 + \frac{C}{100}\right)^2$, both in percentage.

VALUES OF C AND f(C) FOR SUPPLEMENTARY VALUES OF n.

	=	150	200	250	300	350	400	450	500	550
	=	9.4	8.8	8.3	8.0	7.7	7.5	7.3	7.1	7.0
(C)	=	2.1	2.0	1.9	1.8	1.7	1.7	1.6	1.5	1.5
	=	600	650	700	750	800	850	900	950	1000
	=	6.8	6.7	6.7	6.6	6.5	6.4	6.4	6.3	6.3
(C)	=	1.5	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4

GENERAL DIRECTIONS

$$I_n''' = \frac{\text{mean of } (3 \text{ max.} — 3 \text{ min.})}{A = \text{average of n values}}\,; \quad I_n' = \frac{\text{max.} — \text{min.}}{A}$$

$${}_3I_n' = I_n''' + \left(\frac{C}{100}\right) I_n''', \text{ if } I_n''' < 2.8$$

$${}_3I_n' = \left[1 + \frac{I_n'''}{4}\left(\frac{C}{100}\right)\right]\left[1 + \frac{C}{100}\right]I_n''',$$

if $I_n''' < 2.8$

The following formula gives a closer approximation

$${}_3I_n' = I_n''' + \frac{C}{100}\, I_n''' + \frac{1}{100}\, f(C)\, (I_n''')^2$$

where C and the function of (C) are tabulated in per cent.

TABLE 6

* MEDIAN PERCENTAGE TO {add or subtract} FOR REDUCING THE PRECIPITATION RATIO FOR n YEARS TO THAT OF 50 YEARS AND PROBABLE LIMITING VALUES (in brackets).

n years	0	1	2	3	4	5	6	7	8	9	
0						93 (56—155)	75 (48—133)	64 (42—107)	57 (37—100)	51 (33—89)	*Add*
10	47 (30—78)	43 (28—73)	40 (26—67)	36 (24—60)	33 (22—55)	30 (20—50)	28 (19—47)	26 (18—44)	24 (17—41)	22 (15—38)	
20	21 (14—35)	20 (14—33)	19 (13—31)	17 (12—29)	16 (11—27)	15 (9—25)	14 (9—23)	14 (8—21)	12 (8—19)	11 (8—18)	
30	11 (7—17)	10 (7—16)	9 (7—15)	8 (6—14)	8 (6—13)	7 (5—12)	6 (5—11)	5 (4—10)	5 (4—9)	4 (3—8)	
40	4 (3—7)	3 (2—6)	3 (2—5)	3 (2—4)	2 (2—3)	2 (1—3)	2 (1—3)	2 (1—3)	1 (1—2)	1 (1—1)	
50	0 0	1 (1—1)	1 (1—1)	2 (1—3)	2 (1—3)	3 (1—3)	3 (2—3)	3 (2—4)	+3 (2—4)	+4 (2—4)	*Sub-tract*
60	+4 (2—5)	+4 (2—5)	+4 (2—6)	+5 (3—6)	+5 (3—6)	+5 (3—7)	+6 (3—7)	+6 (4—8)	+6 (4—8)	+6 (4—9)	
70	+6 (4—9)	+6 (4—9)	+6 (4—10)	+6 (4—10)	+7 (5—11)	+7 (5—11)	+7 (5—11)	+7 (5—11)	+7 (5—12)	+7 (6—12)	
80	+7 (6—13)	+7 (6—13)	+7 (6—13)	+7 (6—13)	+7 (6—14)	+7 (6—14)	+7 (6—14)	+7 (7—14)	+8 (7—14)	+8 (7—15)	
90	+8 (7—15)	+8 (7—15)	+8 (7—15)	+8 (7—16)	+8 (8—16)	+9 (8—17)	+9 (8—17)	+9 (8—17)	+9 (8—18)	+9 (8—18)	
100	+10 (8—18)										

---

* These percentages are theoretical values using assumptions that give results agreeing closely with observed precipitation ratios based upon the following six groups of stations:

Median Percentage to (add or subtract) for reducing the precipitation ratio, etc.—

	SOUTHERN CALIFORNIA	Precipitation Index for 50 yrs.
I	El Cajon, Escondido, Poway	1.2 - 1.4
II	El Cajon, Elsinore, San Bernardino	1.3 - 1.7
III	Santa Monica, San Diego, Bonita, Chula Vista, Southeastern desert of California	1.3 - 1.6
IV	Bagdad, Barstow, Blythe, Indio, Parker	1.9 - 5.0
V	Pacific Islands:	
VI	Arabian Desert:	2 - 4

Factors for standardizing the precipitation ratio to a fifty-year interval.

REGIONS—Regions designated by Roman Numerals*

I *California Stations*
Poway, El Cajon, Escondido

II *California Stations*
Santa Monica, San Diego, Bonita, Chula Vista

III *European Stations*
San Fernando, Modena, Paris, Geneva, Culloden

IV *Pacific Island Station*
Banaba

V *Pacific Island Stations*
Apia, Honolulu

VI *California Stations—(Desert)*
Bagdad, Barstow, Blythe, Indio, Parker

VII *Portugal*
Lisbon

VIII *U. S. A. Station*
Philadelphia

IX *California Stations*
Avalon, Los Angeles, Point Loma

X *California Stations—(Desert)*
Greenland Ranch, Independence, Mina, Palm Springs

XI *Desert Stations*
California—Amos, Mojave, Needles
Nevada—Las Vegas
Arizona—Yuma

XII *Arabian Desert Stations*
Basra, Bagdad, Muscat, Port Said, Ghardia, Suez Canal

TABLE 7

FACTORS FOR STANDARDIZING THE PRECIPITATION RATIO TO A FIFTY-YEAR INTERVAL

(Under each line of theoretical factors, designated by letters corresponding to Table I, are entered factors based upon observations at selected stations indicated by Roman numerals listed in the first column.)

Years		5	10	15	20	25	30	35	40	60	70	80	100
Regions*	Designation of theoretical factors in Table I												
	A	1.56	1.30	1.20	1.14	1.09	1.07	1.05	1.03	.98	.96	.94	.92
I				1.18	1.13	1.10	1.07	1.04	1.03	.98	.96	.95	.90
	C	1.93	1.46	1.29	1.20	1.14	1.10	1.07	1.04	.97	.95	.93	.90
	E_1	1.96	1.47	1.30	1.21	1.15	1.11	1.07	1.04	.97	.95	.93	.90
II				1.25	1.18	1.13	1.10	1.06	1.03	.97	.94	.92	.88
III				1.27	1.19	1.14	1.10	1.06	1.03	.97	.94	.92	.88
IV		1.69	1.40	1.27	1.20	1.14	1.10	1.07	1.04	.96	.94	.92	.89

	E_3	2.04	1.52	1.33	1.23	1.16	1.11	1.08	1.05	.96	.94	.92	.88
V		1.88	1.49	1.32	1.22	1.16	1.11	1.07	1.05	.96	.94	.92	.88
III				1.27	1.19	1.14	1.10	1.06	1.03	.97	.94	.92	.88
VI				1.31	1.22	1.16	1.11	1.07	1.04	.96	.94	.91	.88
VII				1.39	1.27	1.19	1.14	1.08	1.05	.96	.92	.90	.86
VIII				1.36	1.25	1.18	1.13	1.08	1.05	.96	.93	.91	.87
IX				1.38	1.27	1.19	1.13	1.08	1.05	.96	.92	.90	.86
	E_4	2.13	1.57	1.37	1.25	1.18	1.13	1.09	1.05	.96	.93	.91	.87
	†	2.09	1.55	1.36	1.24	1.19	1.13	1.09	1.06	.97	.94	.92	.87
	E_5	2.24	1.63	1.40	1.28	1.20	1.14	1.09	1.06	.96	.92	.90	.86
VIII				1.36	1.25	1.18	1.13	1.08	1.05	.96	.93	.91	.87
VII				1.39	1.27	1.19	1.14	1.08	1.05	.96	.92	.90	.86
X				1.40	1.28	1.20	1.14	1.09	1.05	.96	.93	.90	.85
IX				1.38	1.27	1.19	1.13	1.08	1.05	.96	.92	.90	.86
	E_7	2.25	1.78	1.50	1.35	1.25	1.17	1.12	1.07	.95	.91	.87	.82
XI				1.48	1.33	1.23	1.16	1.10	1.06	.95	.92	.88	.84
XII				1.50	1.34	1.24	1.17	1.11	1.06	.95	.91	.88	.84

---

*Average values of factors found empirically from representative observations adopted as provisional values for use in climatological researches.

LITERATURE CITED

Birkeland, B. J.

1935. Mittle and Extreme Der Lufttemperatur. Mit 4 Textfiguren. Geofysiske Puulikasjoner, vol. 14, no. 1.

Commissopulos, N. A.

1921. Variability of Mean Annual Temperature Over North America and Europe During the Ten Years 1900-1909. Royal Meteor. Soc. Quarterly Journal 47, pp. 283-286.

Dodd, Edward Lewis

1923. The Greatest and Least Variate Under General Laws of Error. Reprinted from the Transactions of the American Mathematical Society, vol. 25, no. 4, pp. 525-539.

Irwin, J. O.

1925. On a Criterion for the Rejection of Outlying Observations. Biometrika 17, pp. 238-250.

1925. The Further Theory of Francis Galton's Individual Difference Problem. Biometrika 17, pp. 100-128.

Kondo, T.

1930. A Theory of the Sampling Distribution of Standard Deviations. Biometrika 20, pp. 36-64.

1930 Table of Normal Curve Functions to Each Permille Frequency. Revised by Ethel M. Elderton. Biometrika 20, pp. 368-376.

Pearson, Karl

1930. Tables for Statisticians and Biometricians. Part I, 3rd Edition. Issued by the Biometric Laboratory, University College, London.

1931. Tables for Statisticians and Biometricians. Part II, 1st Edition. Issued by the Biometric Laboratory, University College, London.

Pearson, Egon S.

1932. The Percentage Limits for the Distribution of Range in Samples from a Normal Population. Biometrika 24, pp. 404-417.

Simpson, George Gaylord

1941. Range as a Zoological Character. American Journal of Science, vol. 239, no. 11, pp. 785-804.

Thielebein, Heinrich

1933. Reduktionsfaktoren zum Hellmannschhen Schwankungsquotienten. Meteorologische Zeitschrift, Band 50, pp. 178-183.

Tippett, L. H. C.

1925. On the Extreme Individuals and the Range of Samples Taken from a Normal Population. Biometrika 17, pp. 364-387.

Whittaker, E. T.
Robinson, G.

1932. The Calculus of Observations. A Treatise on Numerical Mathematics. 2nd Edition.

Williamson, A. V.
Clarke, K. G. T.

1931. The Variability of the Annual Rainfall of India. Royal Meteor. Soc. Quarterly Journal 57, pp. 43-56.

Wright, Sewell

1926. A Frequency Curve Adapted to Variation in Percentage Occurrence. Journal of the American Statistical Association. vol. 221, nos. 153-156, pp. 162-178.

Yasżkawa, Kazutaro

1925. On the Means, Standard Deviations, Correlations, and Frequency Distributions of Functions of Variates. Biometrika 17, pp. 211-237.

Contribution No. 207

(Reprinted in U. S. A. from *Transactions* of 1943
of the American Geophysical Union)

RESEARCH WITHIN PHYSICAL OCEANOGRAPHY AND SUBMARINE GEOLOGY AT THE SCRIPPS INSTITUTION OF OCEANOGRAPHY DURING APRIL 1942 TO APRIL 1943

H. U. Sverdrup and Staff

(Contributión from the Scripps Institution of Oceanography, New Series, No. 207)

Academic research activity in the fields of physical and chemical oceanography and submarine geology has been further curtailed during the past year due to increased participation of members of the staff in war work. Several members are on special war leave and, since August, 1942, the remaining personnel in physical oceanography and the facilities of the Scripps Institution have been completely at the disposal of special war projects. Consequently, there is little to report to the American Geophysical Union.

G. F. McEwen has temporarily discontinued various research activities in progress with the view of resuming his work when conditions make this possible. The climatology-investigations with Gorczynsky were concluded with the exception of a statistical paper by McEwen, completed later. The usual work on long-range weather forecasting was definitely terminated, and the material placed in storage. It is the belief that the Government investigations in this field, brought about by increased demands, will lead to a use and expansion of Government facilities and personnel in this field somewhat comparable to the complexity and importance of the problem. Work on the problems of dispersion and turbulence was stopped, and the material indexed and filed. The material on computing surface-currents from sea-surface temperatures was indexed and filed, with the intention of continuing these researches, to a limited extent, along with the war work.

The usual observations of temperature, salinity, and weather at our six shore stations and supplementary observations from United States Lighthouse Tenders have continued. There were about 2200 salinity observations, 2200 sea-temperature observations, and 1500 wind observations. Continuous barograph and pyrheliometer records and daily observations of humidity and precipitation were maintained at the Scripps Institution.

Under the direction of E. G. Moberg, J. C. Hindman has nearly brought to a conclusion the investigation begun last year on equilibria in the system calcium carbonate-carbon dioxide-water in salt solutions and sea-water. This work was undertaken with a view of gaining further information regarding the much-investigated problem of the solubility of calcium carbonate in sea-water at various temperatures, salinities, carbon-dioxide tensions, and, indirectly, hydrostatic pressures. The essential procedure consisted of equilibrating, at various temperatures, natural sea-water samples in a thermostat with solid calcium carbonate, the equilibrium being approached both from undersaturation and supersaturation with respect to calcium carbonate. When equilibrium had been established the carbonate ion-content was determined by electrometric titration with a standard acid, using the most advanced types of glass electrodes, a high-grade potentiometer, and a sensitive electron-tube voltmeter previously developed and described by L. J. Anderson and J. C. Hindman (Ind. and Eng. Chem., v. 15, pp. 42-45, 1943). With this apparatus assembly, hydrogen-ion concentration values accurate to pH 0.01 or better were obtained. In a number of experiments various types of salt solutions were substituted for natural sea-water in order to ascertain the possible effect on the calcium equilibrium of the minor substances, especially organic matter, contained in sea-water. The data obtained from this study are now being analyzed and no final conclusions can as yet be drawn. It can be stated, however, that, in agreement with most previous investigators, surface sea-water is supersaturated with respect to calcium carbonate, that equilibrium is established very slowly, and that the latter fact, together with the use of faulty methods or erroneous chemical constants, probably accounts for the great divergencies in the previously published data.

Since last year additional experimental studies have been made of a method for determining the chlorinity of sea-water more precisely than is possible with routine methods, and a paper describing it is now in press (J. Amer. Chem. Soc.). The method is intended for use in the preparation of ''standard'' sea-water and in investigations requiring greater accuracy than attainable with the usual more rapid methods. It differs from the Knudsen chloride titration method in that both the sample and the titrant ($AgNO_3$) are measured gravimetrically rather than volumetrically, and that the titration end-point is determined electrometrically, using silver-silver chloride electrodes and the Anderson-Hindman voltmeter. The resulting increase in precision is about tenfold.

Several of the reports dealing with results of the E. W. Scripps cruise to the Gulf of California in 1940 have been completed and one is nearing completion. C. A. Anderson, Professor of Geology, University of California at Berkeley, has submitted his manuscript on the geology of islands and neighboring land areas for publication by the Geological Society of America. Similarly, J. W. Durham of the International Petroleum Company has submitted his manuscript on megascopic paleontology and marine stratigraphy to the Geological Society of America. Within the next month F. P. Shepard expects to complete a manuscript on the submarine topography of the Gulf of California, which was mentioned in last year's report.

Two books on oceanography by members of the staff, manuscripts for which were completed prior to 1942, have been published as follows:

Oceanography for meteorologists, by H. U. Sverdrup, Prentice-Hall, New York, 250 pages, April, 1942.
The oceans: Their physics, chemistry, and general biology, by H. U. Sverdrup, M. W. Johnson, and R. H. Fleming, Prentice-Hall, New York, 1087 pages, December, 1942.

A new series, Records of observations, Scripps Institution of Oceanography, has been started. The first number of this series, ''Oceanographic observations on the E. W. Scripps cruises of 1938,'' was published in July, 1942, and the next two issues dealing with observations in 1939 and in 1940 can be expected to be ready for distribution in 1943. In the first issue of this series are published tables showing interpolated values of temperature, salinity, and oxygen content at standard depths, and computed values of anomalies of specific volume and dynamic depth, as well as tables showing the number of diatoms at different depths in the surface layers. The next issue will also contain soundings and results of current measurements.

Scripps Institution of Oceanography,
University of California,
La Jolla, California

Contribution No. 208

Reprinted from the Proceedings of the NATIONAL ACADEMY OF SCIENCES,
Vol. 29, No. 8, pp. 225–228. August, 1943

THE STRUCTURE AND FUNCTION OF THE GOLGI SYSTEM IN THE LIVING CELLS OF DEVELOPING MOLLUSCS

BY LEONARD G. WORLEY

DEPARTMENT OF BIOLOGY, BROOKLYN COLLEGE AND THE SCRIPPS INSTITUTION OF OCEANOGRAPHY*

Communicated July 14, 1943

Practically all of our knowledge of the Golgi apparatus has been built up through the study of fixed, osmic or silver, preparations, it not being generally realized that this important cytoplasmic inclusion can be revealed in many living cells by means of vital methylene blue staining. Reliance on the fixed preparations has created a great deal of confusion with regard to the structure of the Golgi bodies, because their nature is such that they only rarely lend themselves to faithful preservation by fixing fluids. In molluscs, these inclusions are of such large size that it has been possible to follow their behavior in intact embryos over periods of several days and considerable new information has been gathered regarding their structure and function.

Such observations reveal that the generalized Golgi body in molluscs is typically a more or less spherical, simple vesicle, possessing a chromophilic, gel-like, relatively lipoidal pellicle covering a relatively more protein, fluid, chromophobic core. The pellicle is thickened over one surface of the sphere forming a bowl-shaped structure which, through the microscope, looks like a crescent (Fig. 1 (*A*)). The relation between the thickened, bowl-shaped portion of the chromophile and the chromophobe is somewhat like the relation that exists between the "crescent" moon and the moon as a whole, except that in the Golgi body a thin layer of chromophilic material completely encloses the chromophobe. These generalized bodies are clearly endowed with the ability to keep the lipoidal and protein components segregated, hence the visible differentiation into chromophile and chromophobe. But they are the immediate descendants of homogeneous droplet-like forms where no such ability is manifest (Fig. 1 (*B*)). These droplets, in turn, are derived from smaller and presumably more solid granules which may be considered "reserve" forms (Fig. 1 (*C*)).

The enlargement of the granules to form droplets and the development of the droplets into larger, simple vesicles are due, with very little doubt, to the absorption of substances from the cytoplasm. This absorbing quality, together with the segregating tendency already referred to, constitute the two most fundamental physiological characteristics of the Golgi substance in these species.

In developing molluscs, the generalized Golgi bodies give rise, following gastrulation, to exceedingly active and highly specialized forms. By this period, quantities of the original yolk have been dissolved in the cytoplasm and although much has, no doubt, been oxidized, the Golgi elements absorb large amounts of the material and increase considerably in size, forming elaborate, compound vesicular bodies (Fig. 1 (*D*)). Within the chromophilic material, new vesicles continually make their appearance and, in the trochophore larva, small (1^{μ}) fat droplets are continually discharged into the cytoplasm. In the pelecypod Mytilus, the absorption by the Golgi bodies of all of the ultramicroscopic, pigmented fat particles of the single-celled egg results in the freeing of the ground cytoplasm of pigment and the deposition of this pigment in the Golgi inclusions.

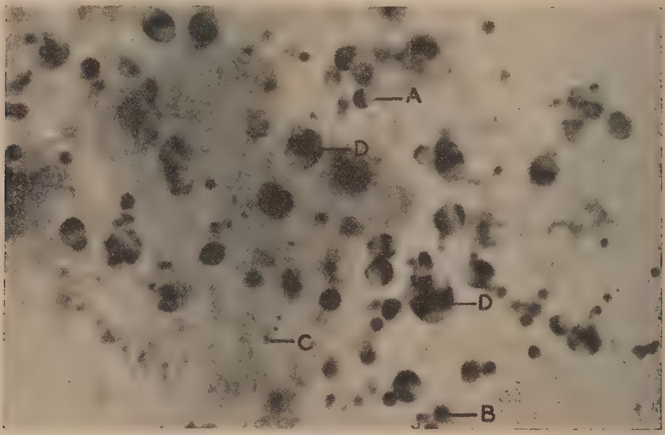


FIGURE 1

Living Golgi bodies seen in trochophore of the tectibranch Navanax (× 1000). *A*, generalized form, showing "crescent"-like chromophile, and chromophobe; *B*, droplet; *C*, granule; *D*, compound vesicular forms, each showing several chromophobes.

The discharge from each enlarged Golgi body of large ($4–6^{\mu}$) protein spheres (the original chromophobes) is characteristic, in nudibranchs and tectibranchs, of the early veliger larva. In this process, the chromophilic portion of the structure withdraws from around the chromophobe and condenses into a chromophilic, pycnotic mass, which can be shown to be rather solid. The pycnotic phase, however, is soon followed by a stage in which the mass becomes resolved into a number of spherical, relatively fluid, droplets. Each of these soon differentiates into a generalized vesicle as the segregating tendency is resumed and the cycle of the system begins anew.

The breaking up of the pycnotic Golgi body into droplets is the reproductive phase of the cycle and is the only method by means of which the Golgi elements increase in number during early development. In other words, there is no "dictyokinesis" accompanying every division of the cells, all multiplication being a mass division of the sort just described and always occurring after the assumption of a pycnotic condition following a period of marked synthetic activity. This also appears to be the situation during oögenesis where the synthetic period results in the deposition of large quantities of fat and protein yolk in the oöcyte. The multiplication period clearly increases the number of Golgi elements, thereby in tectibranchs providing a sufficient number for the new cells of the growing embryo. In the event that the embryo is not increasing in size during this period, as in Mytilus, this increase in number may be offset by a fusion phase preceding the active synthetic period. The fusion phase reduces the total number of Golgi bodies, but the original number is approximately restored during the multiplication period.

In the account just given, the internal chromophobe of each Golgi body is a potential protein or fat product which gradually becomes much more viscous as development proceeds. The elaboration of such products, in molluscs, occurs chiefly *within* the Golgi vesicle, but elaboration *outside* the vesicle is employed in Mytilus in the development of certain large oily yolk droplets. In this case, the product first makes its appearance within a group of small Golgi bodies, all of which appear to contribute to the single common yolk sphere. The mechanism involved in this process is not clear, but it is significant that elaboration of a product can take place merely through contact of the Golgi body with the ground cytoplasm.

In developing molluscs, the function of the Golgi system appears to be the continual mobilization of the fat and protein reserves within the cell. Why the embryo should continually dissolve its formed yolk inclusions, only to elaborate new ones is not immediately evident, but that the phenomenon is of fairly general occurrence in animals is indicated by the work of Schoenheimer[1] who finds that almost all of the proteins of the body are continually undergoing synthesis and breakdown. Should the Golgi system be found to be concerned in this protein turnover in higher animals, as it seems to be in the molluscs, it would prove to be a cytoplasmic constituent of greater metabolic significance than we have realized.

Summary.—For the first time, the Golgi system has been continuously followed in living, developing animal eggs. The multiplication of the Golgi elements has been observed and the rôle of the Golgi substance as an absorbing mechanism and protein and fat elaborating system is described. The active Golgi bodies are found to originate from minute, chromophilic "reserve" granules.

---

* Contribution from the Department of Biology, Brooklyn College, No. 45, and Contribution from The Scripps Institution of Oceanography, New Series No. 208.

[1] Schoenheimer, R., *The Dynamic Body Constituents*, Cambridge, Mass., 1942.

Contribution No. 209

Reprinted from the Proceedings of the NATIONAL ACADEMY OF SCIENCES,
Vol. 29, No. 8, pp. 228–231. August, 1943

THE RELATION BETWEEN THE GOLGI APPARATUS AND "DROPLETS" IN THE CELL STAINABLE VITALLY WITH METHYLENE BLUE

By LEONARD G. WORLEY

DEPARTMENT OF BIOLOGY, BROOKLYN COLLEGE AND THE SCRIPPS INSTITUTION OF OCEANOGRAPHY*

Communicated July 14, 1943

There has been considerable difference of opinion concerning the nature of certain "droplets" that become visible in cells when the tissue is stained with various basic vital dyes. One group of workers, notably Beams,[1] Ludford,[2] Chang[3] and others, holds that these are "neoformations" resulting from the vital dye treatment and that they have no counterpart in the unaltered, living cell. Another group of investigators, notably Covell and Scott,[4] Cowdry and Scott,[5] Ma,[6] Owens and Bensley[7] and Parat,[8] are of the opinion that these "dye droplets" represent the living Golgi material and that the classical Golgi apparatus of the fixed and osmicated cell results either from a precipitation of the osmic salt in and around these droplets, or from their running together and fusion as the result of fixation. In recent years, the tendency has swung in favor of the first contention, but crucial proof of neither of these views is at hand.

In my work, "droplets" of this kind have been revealed by means of supra- or intravital methylene blue staining of the tissues of many invertebrates, including the salivary gland cells of the larva of the midge Chironomus, and the smooth muscle, pancreas, liver and gall bladder of the frog and the smooth muscle, adipose tissue, pancreas, liver, kidney, thymus, bladder and pars distalis of the kitten. Usually, these "droplets" are opaque and show, in life, no internal structure and the reasons for their being considered "dye droplets" by so many workers are evident.

However, in all cases thus far examined, when living tissues containing these "dye droplets" are treated with hypertonic salt solution, thus simulating the shrinkage that so frequently attends osmic acid fixation, the "droplets" rupture and form in each cell a group of fixtures that collectively so closely resemble the classical Golgi apparatus that there is little doubt but that the two are identical.

Experiments with hypertonic salt solution have shown that each "excretion droplet" is in reality a duplex structure, consisting of a rather thick, chromophilic, gel-like pellicle and a fluid core, the latter corresponding to the chromophobe or osmiophobe of many invertebrate Golgi bodies. The fluid interior appears to be held under some slight pressure by the pellicle and the structure, far from being a mere droplet of dye is actually a simple turgid vesicle.

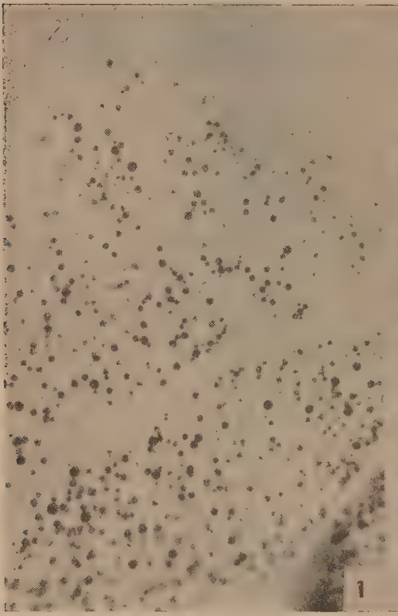


FIGURE 1

Living Golgi vesicles in the salivary gland of the larva of Chironomus, supravitally stained with methylene blue (× 500).

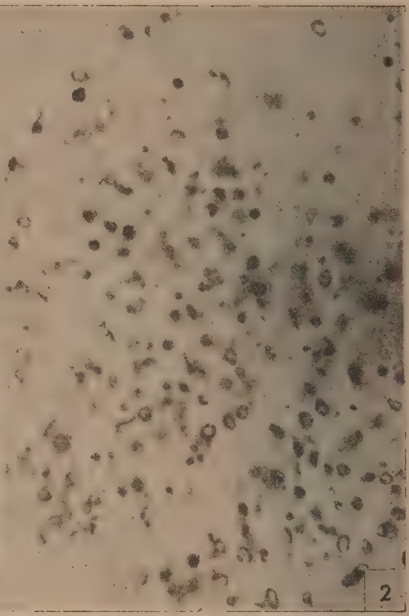


FIGURE 2

Same five minutes later after treatment with hypertonic salt solution (× 1000). The vesicles have ruptured, producing the classical Golgi figures. Compare with figures of Beams and Goldsmith.[9]

When these Golgi vesicles in the relatively inactive gland cells of Chironomus (Fig. 1), for example, are treated with hypertonic salt solution, many chromophilic rings are immediately formed, each of which usually shows a localized thickening (Fig. 2). These vesicles, therefore, have an internal structure which no ordinary dye droplet could possibly be claimed to possess. Furthermore, the pellicle is revealed by these experiments to be much thicker than would be the case of a surface membrane that might conceivably be accredited to an excretion droplet. When relatively active gland cells are similarly treated, the fluid vesicle content squirts out into the cytoplasm forming chromophilic "streamers" which immediately solidify, or the entire vesicle may elongate forming a coarse thread. Under favorable circumstances, these threads and streamers, in the mammalian acinar cells of the pancreas, contact each other forming a chromophilic pseudoreticulum which bears a striking resemblance to the classical Golgi apparatus of the osmicated cell. This resemblance may be further increased by allowing the material to desiccate on the slide, thus simulating tissue dehydration. Frequently, both intact, and ruptured or elongated, vesicles may occur side by side in the same cell. This probably accounts for the widespread belief that since "dye droplets" or "vacuome (?)" (unruptured Golgi vesicles) and Golgi apparatus (ruptured or elongated Golgi vesicles) can be demonstrated in the same cell, the two are not identical. Indeed, this may be true in some tissues, but it is certainly not the case in those mentioned above.

When vitally stained amphibian smooth muscle cells are treated with hypertonic salt solution, the Golgi vesicles become flattened into discs which, upon desiccation, due to their soft gel-like consistency often fuse to form a longitudinal plate-like structure. It is probably in this manner that the classical lamellar form of the fixed Golgi apparatus in these cells is brought about by the fixing fluid. When vitally stained tissue is treated with hypertonic salt solution after a considerable delay following its removal from the animal, distortion of the vesicles is less marked, probably because on standing the vesicles undergo a considerable increase in viscosity.

These statements are not intended to imply that true excretion droplets never occur in vitally stained tissues, but it is perfectly clear that in the tissues thus far examined, the classical Golgi apparatus of the fixed cell is formed from chromophilic vesicles of the living cell that have frequently been considered "neoformations." It appears very probable that there is a correspondence between the "vacuome" and the Golgi system in far more instances than we have been accustomed to believe. At the same time, the vital methylene blue staining technique has revealed true Golgi puddingstone-like masses in invertebrate oöcytes and developing embryos which, in section, appear network-like.

Summary.—The living Golgi system in many invertebrate and vertebrate cells consists of a series of chromophilic vesicles, each with a rather thick gel-like pellicle enclosing a fluid core. Upon treatment of the vitally stained tissue with hypertonic salt solution, thus simulating fixation shrinkage, these vesicles can be observed to collapse, discharge the fluid vesicle content or elongate forming a pseudoreticulum similar to that exhibited by the Golgi apparatus of the osmicated cell. Desiccation, superimposed upon the shrunken material, provides an even more striking picture of the classical Golgi apparatus.

* Contribution from the Department of Biology, Brooklyn College, No. 46, and Contribution from The Scripps Institution of Oceanography, New Series No. 209.

[1] Beams, H. W., *Anat. Rec.*, **45**, 137–162 (1930).

[2] Ludford, R. J., *Proc. Roy. Soc. London*, **B107**, 101–114 (1930).

[3] Chang, H. C., *Anat. Rec.*, **62**, 95–104 (1935).

[4] Covell, W. P., and Scott, G. H., *Ibid.*, **38**, 377–400 (1928).

[5] Cowdry, E. V., and Scott, G. H., *Arch. de l'Institut Pasteur de Tunis* (1928).

[6] Ma, W., *Chinese J. Physiol.*, **4**, 381–386 (1930).

[7] Owens, H. B., and Bensley, R. R., *Am. J. Anat.*, **44**, 79–110 (1929).

[8] Parat, M., *Arch. d'Mik. Anat.*, **24**, 73–357 (1928).

[9] Beams, H. W., and Goldsmith, J. B., *J. Morph.*, **50**, 497–516 (1930).

Contribution No. 210

Reprinted for private circulation from
THE BULLETIN OF THE AMERICAN ASSOCIATION OF PETROLEUM GEOLOGISTS
Vol. 27, No. 9, September, 1943

MARINE MICROÖRGANISMS WHICH OXIDIZE PETROLEUM HYDROCARBONS[1]

CLAUDE E. ZOBELL,[2] CARROLL W. GRANT,[3] AND HERBERT F. HAAS[2]
La Jolla, California

ABSTRACT

Bacteria are found in the sea which are capable of utilizing petroleum ether, gasoline, kerosene, lubricating oils, crude oils, petrolatum, paraffine wax, mineral oil, methane, pentane, hexane, decane, trimethylpentane, tetratriacontane, benzene, xylene, toluene, cyclohexane, anthracene, naphthalene, and other hydrocarbons. Sea water from the euphotic zone contains 10 to 1,000 hydrocarbon-oxidizing bacteria per liter, and 100 to 100,000 such bacteria have been found per gram of recent marine sediments. All samples of sediments regardless of distance from land, water depth, or core depth have shown the presence of hydrocarbon-oxidizing bacteria. Most of the bacteria oxidize hydrocarbons only in the presence of free oxygen although some of them can utilize nitrate as a hydrogen acceptor. Possibly some of them can activate sulphate as a hydrogen acceptor. Hydrogen sulphide in concentrations exceeding 0.0001 mol per liter inhibits the bacterial oxidation of petroleum hydrocarbons.

Within certain limits long-chain hydrocarbons are oxidized more readily than those of smaller molecular weight, and aliphatic compounds are more susceptible to bacterial oxidation than cyclic or aromatic hydrocarbons. Compounds with unsaturated bonds are attacked more readily than saturated compounds. Carbon dioxide, methane, organic acids, and bacterial protoplasm are among the products resulting from the bacterial utilization of petroleum hydrocarbons. Species of *Proactinomyces, Actinomyces, Pseudomonas, Micromonospora, Mycobacterium,* and possibly other genera of marine microörganisms are able to oxidize hydrocarbons.

Although the reaction will take place in an aqueous system, the presence of sand, silt, diatomaceous earth, and other inert adsorbents accelerates the bacterial oxidation of hydrocarbons. Samples of crude oil added to marine sediments are rapidly destroyed under aerobic conditions. The activity of hydrocarbon-oxidizing bacteria, which appear to be widespread in occurrence, may account for the failure of certain workers to find petroleum hydrocarbons in sediment samples. Similarly such bacteria might prevent the accumulation of detectable quantities of hydrocarbons in experiments with mixed cultures designed to demonstrate the transformation of organic matter into hydrocarbons. Our work suggests that one might expect to find oil accumulating in recent sediments or in experimental material only if conditions are inimical to the activity of hydrocarbon-oxidizing bacteria.

ACKNOWLEDGMENTS

The writers wish to acknowledge the constructive criticisms of L. W. Blau, B. T. Brooks, Ben B. Cox, G. C. Gester, K. C. Heald, and A. I. Levorsen, who thereby aided in the preparation of this paper. The technical assistance of Barbara F. Brown, Sally Grant, and G. D. Novelli is also acknowledged.

---

[1] Contribution from the Scripps Institution of Oceanography, New Series No. 210 and paper No. 1 from Project 43A sponsored by the American Petroleum Institute. Manuscript received, February 2, 1943.

[2] Scripps Institution of Oceanography, University of California.

[3] Scripps Institution of Oceanography, University of California. On sabbatical leave from Brooklyn College, New York. Assisted by Grant No. 555 from the American Philosophical Society.

INTRODUCTION

Following the isolation and description of bacteria which oxidize methane to carbon dioxide and water (Söhngen, 1906), the work of Giglioli and Masoni (1911, 1917) showed that methane-oxidizing bacteria abound in manure, ditch mud, sewage, and other environments where methane is being produced. Bacteria which attack higher hydrocarbons have been found in soil by Söhngen (1913), Wagner (1914), Tausz and Peter (1919), Büttner (1926), Gray and Thornton (1928), Stone *et al.* (1942), and others.

Although they may not require hydrocarbons for their growth, bacteria which can utilize hydrocarbons seem to occur most abundantly in oil-soaked soil (Baldwin, 1922; Tauson, 1929; Haas *et al.*, 1941; Johnson *et al.*, 1942). Hydrocarbon-oxidizing bacteria have also been found in crude oil by Lipman and Greenberg (1932), Tauson and Shapiro (1934), Wakengut (1936), and Bushnell and Haas (1941), and in storage-tank water by Thaysen (1939) and Bushnell and Haas (1941). Additional literature on the occurrence and activity of hydrocarbon-oxidizing bacteria has been summarized by Hessel (1924), Tauson (1929), and Haas (1942).

Judging from the extensive occurrence of hydrocarbon-oxidizing bacteria in other environments, one might expect to find such bacteria in the sea and particularly in unconsolidated sedimentary materials. If present and functional, such bacteria might preclude the possibilities of finding hydrocarbons in detectable quantities even if the hydrocarbons are being deposited or produced by the transformation of organic matter. Because of the important rôle that they may play in the problem of petroleum genesis, an extensive investigation is being made on the factors which influence the distribution and activity of hydrocarbon-oxidizing bacteria in the sea. The following report summarizes some preliminary observations.

EXPERIMENTAL METHODS

The disappearance or emulsification of petroleum hydrocarbons, the growth of bacteria in mineral media containing hydrocarbons as the only source of energy, and the estimation of the amount of oxygen consumed or carbon dioxide produced are the methods most commonly used to study the activity of hydrocarbon-oxidizing bacteria. We have found oxygen consumption, occasionally supplemented by other methods, most suitable for obtaining information on the occurrence and behavior of hydrocarbon-oxidizing bacteria in the sea.

To demonstrate the presence of hydrocarbon-oxidizing bacteria, aseptically collected sea water was thoroughly shaken to insure uniformity in its composition including its dissolved oxygen content, after which it was siphoned into 60-ml. glass-stoppered bottles. Then 0.1 ml. of sterile liquid hydrocarbon was introduced with a pipette while the bottle was being held in such a way that the hydrocarbon would be entrapped in the shoulder of the bottle until the glass stopper could be inserted. The dissolved oxygen content of duplicate pairs of such bottles was determined immediately using the Winkler technique, and the others were analyzed after 10 to 20 days of incubation in a water bath at 22°C. Sterilized water was bottled in the same way to serve as a control.

The protocol of a representative experiment is summarized in Table I. The fact that considerably more oxygen was consumed in the bottles filled with raw (unsterilized) sea water treated with hydrocarbons than in sterile controls or in the raw sea water to which no hydrocarbon was added indicates that hydrocarbon-oxidizing bacteria were present in the raw sea water. Oxygen consumption was accompanied by a marked increase in the bacterial population as shown by plate counts. From the plates of nutrient medium hydrocarbon-oxidizing bacteria could be isolated.

TABLE I

Amount of Oxygen Consumed in Raw (Unsterilized) and Sterile Sea Water in Presence of 0.15 Per Cent of Different Petroleum Hydrocarbons after 20 Days Incubation at 22°C.

Hydrocarbon	*Sea Water*	*Initial Dissolved Oxygen Content (Mgm./l.)*	*Dissolved Oxygen after 20 Days (Mgm./l.)*	*Oxygen Consumed in 20 Days (Mgm./l.)**
Kerosene	Raw	6.46	0.14	6.32
Kerosene	Sterile	6.27	6.25	0.02
Paraffine oil	Raw	6.52	1.43	5.09
Paraffine oil	Sterile	6.20	6.16	0.04
Crude oil	Raw	6.41	0.00	6.41
Crude oil	Sterile	6.32	6.18	0.14
Petroleum ether	Raw	6.54	0.29	6.25
Petroleum ether	Sterile	6.31	6.23	0.08
None (control)	Raw	6.48	5.02	1.46
None (control)	Sterile	6.26	6.23	0.03

* Mgm./l. (milligrams per liter) is the same as p. p. m. (parts per million).

By use of similar experimental procedures it was found that all 60-ml. samples of sea water collected in the La Jolla region contain bacteria which can utilize kerosene, paraffine oil, crude oil (Kern County), and petroleum ether. The vertical distribution of hydrocarbon-oxidizing bacteria in the sea has not been studied yet. According to ZoBell and Anderson (1936), the bacterial population of the sea decreases rapidly with the depth of the water from a few hundred bacteria per ml. at the surface to fewer than 10 per ml. at depths exceeding 200 meters until the sea bottom is reached. Water from the bottom of the sea ordinarily contains thousands to millions of bacteria per ml., and marine sediments also contain large numbers of bacteria (ZoBell, 1938).

They are most abundant in the topmost portions of cores at the mud-water interface where from 100 to 100,000 hydrocarbon-oxidizing bacteria have been demonstrated per gram of sediment (wet basis). Their abundance decreases exponentially with depth reminiscent of the vertical distribution of bacteria in general (ZoBell and Anderson, 1936). Some have been found in the bottom of the longest core examined, it being about 17 feet in length. The samples were collected from the Channel Island region off the coast of Southern California and from the Gulf of California. The studies include samples collected from water depths as great as 12,370 feet. Large numbers of hydrocarbon-oxidizing bacteria have also been found in mud from Mission Bay (ZoBell and Feltham, 1942), Cardiff Slough, and San Diego Bay.

KINDS OF HYDROCARBONS ATTACKED

Besides kerosene, paraffine oil, crude oil, and petroleum ether which were used in the exploratory experiments, several other petroleum products are oxidized by bacteria isolated from the sea. Gasoline, lubricating oils of different viscosities, Shell-solvent, petrolatum, paraffine wax, and six different samples of California crude oils have been found to promote the multiplication and respiration of marine bacteria. While these substances contain impurities which might promote bacterial multiplication, the disappearance of oil globules and the stoichiometric consumption of oxygen and the production of carbon dioxide indicate that the substances are utilized almost quantitatively. Several chemically pure hydrocarbons have been used with similar results.

Table II shows the amount of oxygen consumed by mixed cultures of marine bacteria in aged sea water treated with approximately 0.1 per cent of different pure hydrocarbons. The sea water was aged for several weeks in the dark to permit bacteria to oxidize the organic matter normally present in recently collected sea water. It was inoculated with an enrichment culture of hydrocarbon-oxidizing bacteria. The experiment shows that all of the hydrocarbons are attacked by bacteria.

TABLE II

OXYGEN CONSUMED BY MIXED CULTURES OF MARINE BACTERIA IN PRESENCE OF DIFFERENT HYDROCARBONS IN AGED SEA WATER AFTER 14 DAYS INCUBATION AT 22°C.

Hydrocarbon	*Initial Dissolved Oxygen Content (Mgm./l.)*	*Dissolved Oxygen after 14 Days (Mgm./l.)*	*Oxygen Consumed in 14 Days (Mgm./l.)*
n-Hexane	6.68	0.00	6.68
Cyclohexane	6.71	6.27	0.44
Benzene	6.64	6.51	0.13
Xylene	6.40	0.00	6.40
Toluene	6.60	3.48	3.12
Anthracene	6.69	6.10	0.59
Naphthalene	6.68	0.00	6.68
Pyridine	6.64	2.66	3.98

Although oxygen is consumed very slowly in the presence of cyclohexane, benzene, and anthracene, repeated experiments prove that these compounds are definitely oxidized by bacteria. There are other factors besides the chemical composition of the various hydrocarbons such as their solubilities and dispersion in sea water which influence the rate at which they are utilized. As elaborated in succeeding pages, experiments with otherwise inert adsorbents are in progress on methods of providing for the comparable dispersion of hydrocarbons in aqueous systems.

Besides the hydrocarbons listed in Table II, it has been found that marine bacteria also oxidize methane, n-pentane, pentene, n-heptane, n-octane, trimethylpentane, n-decane, dotriacontane (dicetyl), tetratriacontane, isoprene, and neoprene. Narrow cuts of paraffine waxes having melting points ranging from 45°C. to 75°C. have also been used. ZoBell and Grant (1942) have found marine bacteria which oxidize the hydrocarbons of natural and synthetic rubber.

It appears that, in general, aliphatic compounds are attacked by bacteria more readily than aromatic or cyclic compounds. Within certain limits the longer the chain, the more readily it is attacked by bacteria. For example, when uniformly distributed in the water on sand grains, paraffine wax (probably C_{40} to C_{46}) is oxidized faster by bacteria than is tetratriacontane (C_{34}), and the latter is oxidized faster than n-heptane (C_7) or n-hexane (C_6). Also, it might be significant that petrolatum and lubricating oils are oxidized faster than kerosene, and kerosene is oxidized faster than gasoline. The oxidizability also seems to be a function of the number of side-chains, iso-octane, or trimethylpentane, being oxidized faster than n-octane. Whether these generalizations will continue to maintain when large numbers of pure hydrocarbons have been tested under truly comparable conditions remains to be seen.

Haag (1926) found that the higher the iodine number of paraffine waxes the more readily they were utilized by mycobacteria, thereby leading him to conclude that unsaturated bonds in molecules are attacked preferentially. This may explain why paraffine wax, petrolatum, and lubricating oils are oxidized faster than saturated compounds such as hexane and heptane as noted. On the other hand impurities in the paraffine wax, petrolatum, lubricating oils, kerosene, and gasoline might influence the results.

In spite of differences in the dispersion of hydrocarbons in aqueous media, Söhngen (1913a) noted that as a rule those having a boiling point above 150°C. were assimilated more readily by soil bacteria than those having a lower-boiling point. Strawinski and Stone (1940) have found that hydrocarbons in the range of 10 to 16 carbon atoms per molecule are attacked more readily than those of smaller weight. Tausz and Peter (1919) described a "Paraffine bacterium" isolated from soil which utilized hexadecane (C_{16}), triacontane (C_{30}) and tetratriacontane (C_{34}) but had little or no effect on the lower paraffines, n-hexane, and n-octane, or on benzene or naphthalene derivatives.

Bacterium aliphaticum (Tausz and Peter, 1919) had no effect on cyclic hydrocarbons, but it attacked all aliphatic compounds tried including n-hexane, n-octane, dimethyloctane, n-hexadecane, triacontane, and tetratriacontane. *Bact. aliphaticum* was unable to attack hexylene but it did attack the higher olefines, n-caprylene and hexadecylene. Certain cultures of Tausz and Peter (1919) utilized cyclohexane and dimethylcyclohexane. Johnson *et al.* (1942) found that *Bact. aliphaticum* grew well on pentane, hexane, heptane, octane, nonane, and dodecane. It grew only slightly on methylcyclohexane and not at all on benzene, toluene, or xylene.

According to Lantzsch (1922), *Actinomyces oligocarbophilus* could assimilate higher aliphatic hydrocarbons but not the lower ones, and it could not assimilate the aromatic compounds, benzene and xylene. However, it is not to be construed that the lower hydrocarbons are not assimilated by any bacteria because the work of Kaserer (1906), Störmer (1908), Münz (1920), Aiyer (1920), and others shows that certain bacteria oxidize methane. Söhngen (1906) isolated and described

Bacillus methanicus which is now called *Methanomonas methanica* (Bergey *et al.*, 1939). Recently G. David Novelli, working in the Scripps laboratories, has demonstrated the presence of methane-oxidizing bacteria in marine sediments.

Goobin (1923) described *Bacterium hidium* which oxidizes ethane, and an ethane-oxidizing bacterium, *Bacillus ethanicus*, is mentioned by Blau (1942). The methane-oxidizing bacterium of Tausz and Donath (1930) also utilizes ethane, propane, butane, and other hydrocarbons higher in the series more readily as the length of the chain increases. This latter generalization applies to most of the cultures observed by Tausz and Donath. They studied another bacterium which could utilize only those hydrocarbons having more than 16 carbon atoms per molecule.

Although cyclic hydrocarbons are more refractory to bacterial attack than aliphatic compounds, many bacteria which utilize aromatic compounds have been described. Tauson (1929) described *Bacterium benzoli*, *Bact. toluolicum*, *Bact. naphthalinicus*, and *Bact. phenanthrenicus* which utilize benzene, toluene, xylene, anthracene, and phenanthracene. *Bacillus hexacarbovorum*, isolated from soil by Störmer (1908), oxidizes toluene and xylene as well as methane. The soil bacteria of Strawinski and Stone (1940) attack naphthalene, biphenyl, alpha-methylnaphthalene, tetralin, tertiary butylbenzene, decalin, n-butylbenzene, and isobutylbenzene, the oxidizability being in the order named. They were unable to develop a culture which was capable of growing on benzene or toluene. Some of their bacteria could grow on cetane, 1-octene, and 2-octene. Tausson (1927) has made a special study of the bacterial oxidation of naphthalene as well as of the bacteria which oxidize phenanthrene (Tausson, 1928b).

The writers have found that although toluene inhibits the growth of most microörganisms, including some of the hydrocarbon-oxidizing bacteria, bacteria occur in marine materials which grow freely in the presence of toluene. Most of the toluene, which is virtually insoluble in water, floats on the surface of the media. Oxygen consumption and an increase in the bacterial population in mineral media containing toluene as the only energy source indicates that toluene is oxidized by certain marine bacteria. Toluene is not attacked as readily as many other kinds of hydrocarbons investigated, and the bacteria which attack it are less abundant in marine materials than those which attack long straight-chain compounds.

Similar observations have been made on phenol. One per cent phenol kills most bacteria in a few minutes at room temperature and 0.1 per cent phenol ordinarily inhibits their growth. However, a few marine bacteria have been observed which multiply in the presence of 1.0 per cent phenol in peptone media. Some of them multiply in mineral media containing 0.1 per cent phenol as the only energy source. Cresol-oxidizing bacteria have also been demonstrated in marine materials.

Gray and Thornton (1928) found bacteria to be widely distributed in soil, which oxidize toluene, phenol, o-cresol, m-cresol, p-cresol, phloroglucinol, and resorcinol. They found species of *Micrococcus*, *Mycobacterium*, *Bacterium*, *Bacillus*, and *Spirillum* which decompose aromatic compounds. Erikson (1941) studied *Micromonospora* species from lake mud, which utilize toluene, phenol, resorcinol, m-cresol, β-naphthol, naphthalene, and paradichlorobenzene. The observation that certain bacteria tolerate or utilize these hydrocarbons or hydrocarbon-derivatives is emphasized because the latter have been erroneously credited with preventing bacterial activity in oil deposits.

KINDS OF MICROÖRGANISMS

Most of the hydrocarbon-oxidizing microörganisms isolated from marine enrichment cultures have been proved to be species of *Proactinomyces*, *Actinomyces*, *Pseudomonas*, *Micromonospora*, or *Mycobacterium*. The writers have isolated four species of *Proactinomyces* from hydrocarbon cultures and, judging from colonial characteristics on plates of nutrient agar, several more species occur in marine materials. *Proactinomyces* are filamentous, mold-like bacteria which are differentiated from *Actinomyces* by the former's inability to form spores in the aerial mycelium. According to Umbreit (1939), many soil *Proactinomyces* are able to oxidize petroleum hydrocarbons. Five to 10 per cent of the microörganisms isolated from marine bottom sediments are *Proactinomyces*, but tests have not been made yet to determine how many of them utilize hydrocarbons.

Very few *Actinomyces* have been found in the sea at stations remote from the possibilities of terrigenous contamination, but those which have been isolated oxidize hydrocarbons. Hydrocarbons are oxidized by many terrestrial *Actinomyces* including *A. elastica* and *A. fascus* (Söhngen and Fol, 1914), *A. oligocarbophilus* (Lantzsch, 1922), *A. chromogenes albus*, *A. bovis*, *A. eppinger*, and *A. trautwein* (Büttner, 1926).

Two new species of *Pseudomonas* which oxidize hydrocarbons have been isolated from the sea. It is believed that several other marine species now under observation will likewise be proved to be *Pseudomonas*. Among the hydrocarbon enrichment cultures from terrestrial sources studied by Haas *et al.* (1941) and Stone *et al.* (1942) *Pseudomonas* species predominated. Bushnell and Haas (1941) found that *Ps. aeruginosa* oxidized hydrocarbons, and Johnson *et al.* (1942) observed the utilization of various petroleum hydrocarbons by *Ps. fluorescens*.

Several species of *Mycobacterium* have been found in marine sediments, at least two of which can utilize hydrocarbons as the sole source of carbon and energy. Söhngen (1913a) found that hydrocarbons are oxidized by *Mycobacterium album*, *M. lacticola*, *M. luteum*, *M. phlei*, *M. rubrum*, and *M. hyalinum*. Baldwin (1922) isolated *M. hyalinum* from soil soaked with crude oil. *Mycobacterium* species were ordinarily present in the cultures isolated from oil-soaked soil by Haas *et al.* (1941). Haag (1927) found that the ability of certain species of *Mycobacterium* to utilize hydrocarbons could be used to differentiate them from other bacteria.

Besides the aforementioned genera, bacterial species belonging to each of the following genera have been credited with the ability to oxidize petroleum hydrocarbons: *Bacillus*, *Bacterium*, *Corynebacterium*, *Micrococcus*, *Sarcina*, *Serratia*, and *Spirillum*. Certain molds are also endowed with the ability to oxidize hydrocarbons (Rahn, 1906). Hopkins and Chibnall (1932) have studied the growth of *Aspergillus versicolor* on higher paraffines, and Tauson (1929) noted the growth of *Aspergillus flavus* and a species of *Penicillium* on hydrocarbons. Tauson (1939) found that several yeasts or yeast-like organisms including species of *Debaryomyces*, *Endomyces*, *Hansenia*, *Monilia*, and *Torolopsis* could utilize petroleum hydrocarbons.

These examples should suffice to show that the ability to oxidize petroleum hydrocarbons is very widespread among microörganisms.

EFFECT OF OXYGEN

Virtually all of the results of the microbial oxidation of petroleum hydrocarbons have been obtained with the microörganisms growing in the presence of free oxygen. Whether there are bacteria which can oxidize hydrocarbons under strictly anaerobic conditions is still unknown although there are indications that the reaction can take place in the presence of nitrate, sulphate,[4] and possibly other hydrogen acceptors.

The writers have noted the growth of bacteria and the reduction of nitrate in mineral media containing various hydrocarbons as the sole source of energy. However, this reaction is of little practical significance in source sediments because, as a general rule, if there is no free oxygen in sediments, there will be no nitrate. In fact, nitrate ordinarily disappears before all of the free oxygen is consumed in marine bottom deposits. Thaysen (1939) and Bushnell and Haas (1941) have noted the reduction of nitrate by hydrocarbon-oxidizing bacteria. According to Tausson (1928) *Bacterium benzoli* utilized about 8 grams of benzene in 42 days in the presence of nitrates.

The occurrence of sulphate-reducing bacteria in crude oil and oil-well brines (Bastin, 1926, Gahl and Anderson, 1928, Ginter, 1930, Bastin and Greer, 1930) has been interpreted by some to suggest that sulphate may provide the oxygen for the oxidation of petroleum hydrocarbons. Bacteria which reduce sulphate in the presence of manufactured gas were reported by Kegal (1940), and there is an anonymous (1938) report that certain kerosene-oxidizing bacteria reduce sulphate. Seliber (1928) studied sulphate-reducing bacteria which could obtain their energy requirements from the decomposition of grease and olive oil. The oxidation of crude oil accompanied by the reduction of sulphate by *Vibrio aestu-*

---

[4] Recently Dr. Haas has demonstrated repeatedly the anaerobic utilization of paraffine oil, kerosene, decane, tetradecane, and cetane by bacteria growing in sulphate media. Ferrous iron was used in the media to combine with the hydrogen sulphide as fast as it was formed from the reduction of the sulphate. The hydrocarbon was adsorbed on ignited sand in order to make it more available to the anaerobic bacteria which grow at the bottom of the media. The observation has been confirmed by G. David Novelli.

ari has been reported by Maliyantz (1935) as a means of desulphurizing Russian crude oils, but the bacteria might have been utilizing non-hydrocarbon constituents of the crude oils for energy.

In the exploratory experiments conducted by McCoy and Keyte (1934), they observed the bacterial oxidation of crude oil in a sulphate medium under anaerobic conditions. According to Beckman (1926) bacteria from oil-soaked soil grew under both aerobic and anaerobic conditions in a petroleum distillate having a boiling point of 232°C. Baier (1937) envisions sulphate-reducing bacteria growing in the oil-water interface of petroleum deposits where the bacteria might be able to obtain their energy from the oxidation of crude-oil constituents and their oxygen requirements from sulphate, but he admits conclusive evidence is lacking.

In characterizing oil-field waters, Smith (1931) points out that, with few exceptions, waters associated with oil contain little or no sulphate, which he attributes to sulphate reduction. However, this fails to prove that bacteria are responsible for the reduction of the sulphate, and even if they are, fatty acids and other non-hydrocarbon organic constituents may have served as an energy source. Baars (1930) found that while sulphate-reducing bacteria might utilize certain constituents of crude oil, neither *Vibrio desulfuricans* nor *V. rübentschickii* could utilize pure hydrocarbons. None of a limited number of hydrocarbons was utilized by *Vibrio aestuari*, the marine sulphate-reducer studied by Rittenberg (1941).

The writers have many cultures incubating under different conditions in an effort to ascertain if bacteria exist which can obtain their oxygen requirements from sulphate for the oxidation of petroleum hydrocarbons. Both *Bacterium aliphaticum* and *Vibrio desulfuricans* produce a slight turbidity in sulphate media enriched with either paraffine oil, kerosene, n-hexane, or cyclohexane in an anaerobic environment, but there has been no production of sulphide to indicate sulphate reduction.[4]

It does not seem probable that sulphate can be reduced by hydrocarbon-oxidizing bacteria since, as is elaborated later, hydrogen sulphide is inhibitory. However, the sulphate might be reduced without hydrogen sulphide accumulating in toxic concentrations, the sulphide might be precipitated from solution by heavy metals, or sulphate might be reduced to free sulphur. Conclusions must be withheld until experimental results are available on many more cultures investigated under conditions designed to simulate those which might occur in nature.

Unquestionably free oxygen promotes the activity of the hydrocarbon-oxidizing bacteria which we have isolated from the sea, although the oxygen tension does not seem to be important provided some free oxygen is present. The bacteria begin to grow with equal facility at any oxygen tension tested from a partial oxygen pressure of 12 to 408 mm. of mercury. When only a little oxygen is present, it is quickly consumed after which bacterial multiplication is retarded or stops. If the medium is saturated with oxygen, the hydrocarbon-oxidizing bacteria continue to multiply until all of the oxygen is consumed. This confirms the observations of ZoBell (1940) on the effect of oxygen tension on the rate of oxidation of organic matter by marine aerobes.

EFFECT OF SULPHIDE

The use of enrichment cultures of sulphate-reducing bacteria containing hydrogen sulphide inhibited the oxidation of hydrocarbons by known hydrocarbon oxidizers even under aerobic conditions. This has been attributed to the inhibiting effect of sulphide which is indicated by the data in Table III. Concentrations of sodium sulphide ranging from M/1000 to M/100,000 were added to sea water inoculated with a mixed culture of hydrocarbon-oxidizing bacteria and treated with 0.1 per cent paraffine oil. The oxygen content of the water was determined at the beginning of the experiment and again after different periods of incubation in a water bath at 22°C.

TABLE III

Oxygen Consumed in 21 Days at 22°C. by Marine Bacteria in Sea Water Enriched with 0.1 Per Cent of Paraffine Oil and Treated with Different Concentrations of Sodium Sulphide

Amount of Sodium Sulphide Added (Mols./l.)	*Initial Dissolved Oxygen Content (Mgm./l.)*	*Dissolved Oxygen after 21 Days (Mgm./l.)*	*Oxygen Consumed in 21 Days (Mgm./l.)*
None (control)	6.84	0.03	6.81
0.000,01	7.10	0.88	6.02
0.000,1	7.08	4.73	2.35
0.001	3.26	3.12	0.14

From the data in Table III it may be observed that there was no bacterial activity in the sea water containing M/1000 sodium sulphide, and M/100,000 sodium sulphide was definitely inhibitory. It has been established that the toxic effect is due to the sulphide ion and not to changes in the hydrogen-ion concentration caused by the addition of sodium sulphide. It was necessary to make the water slightly acid in reaction (pH 6.5) by the addition of hydrochloric acid to prevent the sulphide from combining with oxygen. Under these conditions most of the sulphide occurred as hydrogen sulphide. Controls showed that a pH as low as 6.0 does not inhibit marine hydrocarbon-oxidizing bacteria. Hydrogen sulphide is not compatible with oxygen in an alkaline medium.

Whether or not sulphide will be proved to inhibit the oxidation of hydrocarbons under various conditions remains to be seen. According to Elvehjem *et al.* (1941) hydrogen sulphide in concentrations as low as M/2500 inhibits the activity of some oxidases although other oxidases are affected little or not at all. They report that certain oxidases are also inhibited by cyanide, azide, carbon monoxide, fluoride, arsenite, selenite, heavy metals, hydroxylamine, and other compounds. Johnson *et al.* (1942) noted that the bacterial oxidation of octane and nonane was inhibited by 0.000,08 M. cyanide or by 0.25 M. urethane.

The fact that there are substances which inhibit the bacterial oxidation of hydrocarbons suggests that there may be marine environments where hydrocarbons are not attacked by bacteria although such bacteria appear to be widely distributed. On the sea floor one might expect to find significant concentrations of sulphide, carbon monoxide, heavy metals, and maybe other oxidase inhibitors. A detailed study of substances or conditions which inhibit the bacterial oxidation of hydrocarbons is contemplated.

EFFECT OF SURFACE

Most petroleum hydrocarbons are virtually insoluble in water. Consequently they must be attacked by bacteria primarily at the oil-water interface. Anything which tends to increase the dispersion of hydrocarbons in an aqueous system should accelerate their utilization. This has been demonstrated with paraffine wax. When 0.1 gram of wax in one small cube was placed in a bottle of inoculated sea water, 0.32 mgm./l. of oxygen was consumed in 10 days, but when the same quantity of wax was introduced in the form of thin shavings, 1.47 mgm./l. of oxygen was consumed during the same period. When 0.1 gram of paraffine wax was dispersed in the water by mixing 20 grams of ignited sand with molten wax, all of the oxygen (7.46 mgm./l.) was utilized in 10 days.

Attempts have been made to disperse other kinds of petroleum hydrocarbons with the use of biologically inert substances including ignited marine sediment, sand, bentonite, asbestos fibers, and infusorial earth. Table IV illustrates the effect of a sample of marine sediment (a red clay from the Pacific) on the bacterial oxidation of various hydrocarbons. Approximately 20 grams of the sediment (ignited to destroy oxidizable organic matter) were measured into each of several 160-ml. glass-stoppered bottles. Into each bottle 0.1 gram of the hydrocarbon was introduced, and after mixing thoroughly with the dry sediment by shaking, the bottles were filled with inoculated sea water. The results of the experiment are summarized in Table IV.

TABLE IV

Amount of Oxygen Consumed in 10 Days by Bacteria in Aged Sea Water, Part of Which Had Been Treated with Different Petroleum Hydrocarbons, with and without Addition of Ignited Marine Sediment

Hydrocarbon	*Treatment*	*Initial Dissolved Oxygen Content (Mgm./l.)*	*Dissolved Oxygen after 10 Days (Mgm./l.)*	*Oxygen Consumed in 10 Days (Mgm./l.)*
None (control)	None	6.83	6.51	0.32
None (control)	Sediment	6.65	6.13	0.52
Kerosene	None	6.78	2.34	4.44
Kerosene	Sediment	6.70	0.00	6.70
Gasoline	None	6.84	5.29	1.55
Gasoline	Sediment	6.74	0.03	6.71
Benzene	None	6.82	6.36	0.46
Benzene	Sediment	6.71	1.94	4.97
Crude oil	None	6.87	4.56	2.31
Crude oil	Sediment	6.74	0.00	6.74

It may be observed that with each hydrocarbon tested apppreciably more oxygen was consumed by bacteria when the bottles were partly filled with ignited sediment. Since the sediment itself was not oxidizable as shown by the controls to which no hydrocarbon was added, it is concluded that the more rapid utilization of the hydrocarbons is due to their greater availability to bacteria on the surface of the particles of sediment on which the hydrocarbons are adsorbed. Söhngen (1913b) noted that silica or iron oxide stimulated the oxidation of petroleum by *Mycobacterium album*, *Mycobacterium rubrum*, and *Micrococcus paraffinae*. Greig-Smith (1914) found that coating kieselguhr with paraffine accelerated its utilization by *Bacillus prodigiosus* and soil bacteria.

Crude oil is oxidized much more rapidly when mixed with marine mud than in sea water, which is partly attributable to the greater dispersion of the oil in mud and its more intimate contact with bacteria. As much as one gram of Kern County crude oil is quantitatively oxidized in 40 days at 22°C. when mixed with 100 grams of marine mud in a thin layer under aerobic conditions. The bacterial oxidizability of other samples of crude oil is being investigated in different kinds of sedimentary materials and simulated environments. Tauson and Shapiro (1934) found that 45 per cent of Emba crude oil introduced into cultures of bacteria isolated from oil wells was oxidized in seven months and the reaction was still continuing.

TEMPERATURE REQUIREMENTS

Most of the experimental work has been conducted at 22°C., although there are some hydrocarbon-oxidizing bacteria which are active at temperatures as low as 0°C., and others are active at temperatures as high as 55°C. No attempt has been made to grow hydrocarbon-oxidizing bacteria at higher or at lower temperatures. Most of the bacteria isolated from the sea will grow at 0° to 4°C. but very few of them tolerate temperatures exceeding 30°C. (ZoBell and Conn, 1940). This is not surprising when one considers that they have come from an environment having a temperature ranging from 3° to 20°C. There must be other places in nature to find hydrocarbon-oxidizing bacteria which tolerate higher temperatures than those which occur in the sea.

Those bacteria which will tolerate the higher temperatures are much more active than at lower temperatures. For example, seven times as much paraffine wax was oxidized at 55° by a given culture as at 22°C. Although there are relatively few species of marine bacteria which grow at temperatures as high as 55°C., according to Morrison and Tanner (1922), bacteria are quite widely distributed elsewhere in nature which grow at temperatures ranging from 45° to 85°C.

SALINITY REQUIREMENTS

Sea water having a salinity of about 3.4 per cent has been used for the cultivation of the hydrocarbon-oxidizing bacteria. Some of the marine bacteria continue to grow even when sea water is diluted as much as 1 : 10 with distilled water, and most of the hydrocarbon-oxidizing bacteria isolated from the soil grow in very dilute salt solutions. Although only a small percentage of the bacteria isolated from terrestrial or fresh-water sources are able to grow in sea water (ZoBell, 1941), species of *Mycobacterium*, *Pseudomonas*, *Proactinomyces*, and other microörganisms isolated (by Haas) from mid-continental soil, fresh-water wells, sedimentation ponds in oil fields, and "water bottoms" of petroleum-storage tanks have the ability to utilize hydrocarbons in sea-water media.

Marine hydrocarbon-oxidizing bacteria are active in sea water to which 12 to 15 per cent sodium chloride has been added. This represents a four- to five-fold increase in the sodium chloride content of the sea water, sodium chloride being the principal constituent of sea salt. Similar increases in some of the other constituents are not tolerated as well, and it is still unknown if the bacteria are functional in concentrated brines. Studies on the specific salinity requirements of hydrocarbon-oxidizing bacteria are in progress.

The optimum pH for the activity of hydrocarbon-oxidizing bacteria is between 7.5 and 8.0, but they continue to grow until the medium becomes as acid as pH 6.0 or as alkaline as pH 10.

OXIDATION PRODUCTS

Little is known concerning the intermediate and end products resulting from the bacterial oxidation of petroleum hydrocarbons under different conditions. We have noted acid production, the formation of carbon dioxide and, in some cases, methane from the oxidation of paraffine oil and higher petroleum hydrocarbons in sea-water media. The lowered pH may be due to carbonic acid from carbon dioxide. Johnson *et al.* (1942) report that the ratio of carbon dioxide produced to oxygen consumed ranges from 0.47 to 0.63, according to the hydrocarbon undergoing oxidation. This is in agreement with the findings of Stone and co-workers (1942) who give a mean value of 0.65 for the bacterial dissimilation of light oils. They suggest that organic acids may result from the fermentation of hydrocarbons but give no specific information on this point. Hopkins and Chibnall (1932) report that the intermediate products of metabolism of *Aspergillus versicolor* oxidizing higher paraffines are probably ketones or polyketones, and that further oxidation results in the production of shorter fatty acids.

Haas (1942) found no appreciable quantities of intermediate products resulting from the microbial oxidation of petroleum hydrocarbons although in one experiment a substance behaving like isopropyl alcohol was detected. He interprets the $CO_2:O_2$ ratio as indicating that hydrocarbons are more or less completely oxidized to carbon dioxide and water. However, he did find evidence that small amounts of oil-soluble acids were formed. Besides traces of acetic acid and lactic acid, Thaysen (1940) noted the formation of methane, acetaldehyde, and ethane from the bacterial decomposition of kerosene. Hydrogen as well as methane production has also been reported (anonymous, 1938) from the bacterial decomposition of kerosene. Most of the 17 species of hydrocarbon-oxidizing bacteria studied by Söhngen (1913a) produced organic acids as intermediate products.

In a series of experiments designed to test the quantitative assimilation of hydrocarbons, the writers introduced enough n-hexane in glass stoppered bottles of aged sea water with ignited marine sediment to give 2.0 mgm./l. The oxygen content and the bacterial populations of pairs of bottles were determined after different periods of incubation at 22°C. The bacterial population reached a maximum on the fifth day and oxidation appeared to be complete between the fifth and the tenth day. At this time it was found that after subtracting values for the controls, the hexane had accounted for the consumption of 5.16 mgm. of oxygen. Since the complete oxidation of 2 mgm. of hexane to carbon dioxide and water would require 7.26 mgm. of oxygen, at first thought this might be taken to indicate that only 71 per cent of the hexane was utilized. However, direct microscopic counts showed that 14.3×10^9 bacterial cells were formed per liter. Estimating these cells to contain an average of 25×10^{-12} mgm. of carbon each, it follows that 0.36 mgm., or 22 per cent, of the carbon of the hexane was converted into bacterial protoplasm. Similar results have been obtained with tetratriacontane.

DISCUSSION

It is apparent that microörganisms capable of oxidizing petroleum hydrocarbons are rather widespread in sea water and marine sediments as well as on the land. Though not dependent upon hydrocarbons for their multiplication, hydrocarbon-oxidizing bacteria are generally most abundant in oil- or gas-soaked soils. Whereas ordinary soil may contain only a few hundred hydrocarbon-oxidizing bacteria per gram, Haas (1942) has analyzed crude-oil-soaked soil from Texas, Louisiana, and Kansas which contained from a million to 49 million such bacteria per gram. Similarly Baldwin (1922), Tauson (1929), Johnson *et al.* (1942), and others have noted that oil- or gas-soaked soil or water which has been in contact with oil, such as sedimentation ponds or the water bottoms of oil-storage tanks, is the best place to look for hydrocarbon-oxidizing bacteria.

Since, as might be expected, hydrocarbon-oxidizing bacteria are most abundant around oil deposits, the large number of such bacteria found in some samples of marine sediments may indicate the presence of petroleum hydrocarbons. This possibility is being investigated. A method of prospecting for subterranean petroleum deposits has been proposed by Blau (1942) in which oxidation products formed in the surface soil by hydrocarbon-oxidizing bacteria are detected colorimetrically. The method presupposes that volatile hydrocarbons escaping from oil pools are oxidized by soil bacteria in the presence of atmospheric oxygen. Harper (1939) found increased bacterial populations around leaking natural-gas mains. Similar observations were made by Schollenberg (1930). Sanderson's (1942) method of geomicrobiological prospecting is based upon the ability of certain bacteria to utilize volatile hydrocarbons escaping from subterranean deposits.

According to the literature reviewed by Seyer (1933) and Chibnall *et al.* (1934), heptane, pentadecane, hexadecane, eicosane, heneicosane, docosane, tricosane, pentacosane, hexacosane, heptacosane, octacosane, nonacosane, triacontane, pentratriacontane, and other hydrocarbons occur naturally in certain plants and insects which have been examined. In view of the widespread occurrence of petroleum hydrocarbons in the waxes produced by plants, Dean Whitmore at Pennsylvania State College proposed that it may *not be a question of how hydrocarbons are formed but rather a question of what has happened to all of the hydrocarbons which have been produced by plants throughout the geological ages.* Perhaps the hydrocarbons have been destroyed by microörganisms in all except certain environments where conditions are not suitable to the activity of hydrocarbon-oxidizing bacteria. Conditions inimical to the activity of such bacteria may be prerequisite to the accumulation of petroleum hydrocarbons in recent sediments. McCoy and Keyte (1934) and others recognize that hydrocarbon-oxidizing bacteria might destroy or prevent the accumulation of crude oil.

Preliminary observations reported in the foregoing pages suggest that in the absence of free oxygen or in the presence of hydrogen sulphide certain hydrocarbon-oxidizing bacteria may not be active. The demonstrated toxicity of sulphide may be of special significance in light of the belief of many geologists that the richest deposition of source material takes place in euxinic muds which are foul with hydrogen sulphide. Crude oil itself is commonly rich in hydrogen sulphide. Besides hydrogen sulphide and low oxidation-reduction potentials, there may be other influences in certain places on the sea floor such as the presence of carbon monoxide, certain heavy metals or other oxidase inhibitors which protect petroleum hydrocarbons from bacterial oxidation. It would be in such environments where one might expect to find hydrocarbons accumulating.

The activity of hydrocarbon-oxidizing microörganisms may help to explain why Trask (1932) and others have failed to find petroleum hydrocarbons in recent sediments. Even if hydrocarbons were in the sediments *in situ*, they might be destroyed rapidly by the increased bacterial activity and altered environmental conditions which accompany the collection and storage of sediment samples. ZoBell (1938) has shown that following the collection of marine muds, the bacterial population may increase tenfold in a few days when stored at 0° to 4°C., and at higher storage temperatures the increased bacterial activity is proportionately greater. Moreover, if the samples are exposed to air or oxygenated water, there are marked changes in the oxidation-reduction potential, hydrogen sulphide content and other properties of the mud.

Methane was the only hydrocarbon found by Thayer (1931) from the fermentation of fatty acids by mixed cultures of bacteria in marine mud, river mud, sea water, and tapwater. Though the writers have no reason to believe that higher hydrocarbons were produced from fatty acids in Thayer's experiments, it is conceivable some might have been produced and then destroyed by hydrocarbon-oxidizing bacteria, although Thayer (1936) recognizes that certain bacteria utilize hydrocarbons. Be this as it may, it is now apparent that the activities of such bacteria must be taken into account when examining either field or experimental material for petroleum hydrocarbons. In the analysis of 17,400 lbs. of gas resulting from the anaerobic decomposition of sewage sludge Rawn, Banta, and

Pomeroy (1939) detected the presence of 0.04 per cent ethane, 0.01 per cent propane and butane, 0.07 per cent pentane and higher hydrocarbons and 0.001 per cent butadiene along with 67 per cent methane, 32 per cent carbon dioxide, 0.03 per cent carbon monoxide, 0.02 per cent hydrogen and 0.004 per cent hydrogen sulphide.

The observations of Thaysen (1940) and Haas (1942) suggest that bacteria which utilize hydrocarbons might be instrumental in causing undesirable changes in petroleum products stored over water. Thaysen isolated bacteria from the water under kerosene- and gasoline-storage tanks, which decomposed these petroleum products with the formation of methane and possibly ethane. When combined with air these gases could form explosive mixtures which might account for "spontaneous" oil fires. An anonymous (1938) report ascribes the explosion of a kerosene tank to the production of methane and hydrogen by the bacterial decomposition of kerosene in the storage water. The theory is advanced in the report that the bacteria obtained their oxygen from sulphate because sulphate-reducing bacteria were found in the storage water.

Haas (1942) speculates that the discoloration of refinery products and sometimes the formation of gummy substances might be attributable to the activity of hydrocarbon-oxidizing bacteria. He points out that the activity of such bacteria also accounts for the deterioration of paraffine-coated underground cables and for the rapid disappearance of petroleum products which pollute fields and waterways.

CONCLUSIONS

Bacteria are widely distributed in sea water and marine mud which are able to oxidize crude oil, petroleum-refinery products, and many pure hydrocarbons. Several species of bacteria representing five or more genera are endowed with this ability. The hydrocarbon-oxidizing bacteria grow best in the presence of free oxygen, but some of them may be able to obtain their oxygen requirements from nitrate, sulphate or other compounds. Hydrogen sulphide inhibits the activity of aerobic bacteria.

Petroleum hydrocarbons are oxidized more rapidly when dispersed on the surfaces of sand grains, asbestos fibers, diatomaceous earth, or other inert particulate materials. When mixed with 100 grams of marine sediment, as much as one gram of crude oil might be destroyed in 40 days at 22°C. The bacteria grow best at temperatures ranging from 20° to 30°C., although some of them oxidize hydrocarbons at temperatures as low as 0° or as high as 55°C. They grow throughout a wide range of salinity and from pH 6.0 to 10.

All of the bacteria produce carbon dioxide and bacterial protoplasm when they utilize hydrocarbons. Methane, organic acids, ketones, aldehydes, and alcohols have been reported as intermediate products.

It is believed that the presence of large numbers of hydrocarbon-oxidizing bacteria in marine sediments may indicate the presence of proto-petroleum.

However, petroleum hydrocarbons probably will not accumulate in detectable quantities in environments where conditions are favorable for the activity of the bacteria. The failure of various workers to find petroleum hydrocarbons in recent sediments may be due to the increased activity of hydrocarbon-oxidizing bacteria following the collection, transportation, and storage of the samples.

BIBLIOGRAPHY

Aiyer, P. A. S. 1920. "A Methane-Oxidizing Bacterium from Rice Soils," *Mem. Dept. Agr. India*, Chem. Ser., Vol. 5, pp. 173–180.

Anonymous. 1938. "Bacterial Action as a Cause of Spontaneous Oil Fires," *Petrol. Times*, 20, pp. 79–80.

Baars, J. K. 1930. "Über Sulfaatreductie door Bacterien," *Diss. Delft.* 164 pp.

Baier, C. R. 1937. "Bakteriologische Erdölstudien," *Kiel Meeresforschungen*, Vol. 2, pp. 149–56.

Baldwin, I. L. 1922. "Modification of the Soil Flora Induced by Applications of Crude Petroleum," *Soil Sci.*, Vol. 14, pp. 465–75.

Bastin, E. S. 1926. "The Problem of the Natural Reduction of Sulphate," *Bull. Amer. Assoc. Petrol. Geol.*, Vol. 10, pp. 1270–99.

Bastin, E. S., and Greer, F. E. 1930. "Additional Data on Sulphate-Reducing Bacteria in Soils and Waters of Illinois Oil Fields," *ibid.*, Vol. 14, pp. 153–59.

Beckman, J. W. 1926. "Action of Bacteria on Mineral Oil," *Jour. Ind. Eng. Chem.* (News Ed.), Vol. 4, p. 3.

Bergey, D. H., et al. 1939. *Manual of Determinative Bacteriology.* Williams and Wilkins Company, Baltimore. 1032 pp.

Blau, L. W. 1942. "Prospecting for Petroleum Deposits" (U. S. Patent No. 2,269,889). *Chem. Abst.*, Vol. 36, p. 3350.

Bushnell, L. D., and Haas, H. F. 1941. "The Utilization of Certain Hydrocarbons by Microörganisms," *Jour. Bact.*, Vol. 41, pp. 653–73.

Büttner, H. 1926. "Zur Kenntnis der Mykobakterien, insbesondere ihres quantitativen Stoffwechsel auf Paraffinnahrboden," *Arch. f. Hyg.*, Vol. 97, pp. 12–27.

Chibnall, A. C., et al. 1934. "The Constitution of the Primary Alcohols, Fatty Acids and Paraffins Present in Plant and Insect Waxes," *Biochem. Jour.*, Vol.23, pp. 2189–2208.

Elvehjem, C. A., et al. 1939. *Respiratory Enzymes.* Burgess Publishing Company, Minneapolis. 236 pp.

Erikson, D. 1941. "Studies on Some Lake-Mud Strains of Micromonospora," *Jour. Bact.*, Vol. 41, pp. 277–300.

Gahl, R., and Anderson, B. 1928. "Sulphate Reducing Bacteria in California Oil Waters," *Centralbl. f. Bakt.*, Abt. II, Vol. 73, pp. 331–38.

Gainey, P. L. 1917. "Effect of Paraffin on the Accumulation of Ammonia and Nitrates in the Soil," *Jour. Agric. Res.*, Vol. 10, pp. 355–64.

Giglioli, I., and Masoni, G. 1911. "New Observations on the Biological Absorption of Methane, the Distribution of Methane Organisms of Kaserer and Söhngen in the Soils and Fertilizers," *Staz. Sper. Agr. Ital.*, Vol. 42, pp. 589–606.

———. 1917. "New Observations on the Biological Absorption of Methane and the Distribution of Methane Organisms of Kaserer and Söhngen in Soils, Mud and Farm Manures," *R. Univ. Pisa, Inst. Chim. Agraria, Studi Recherche*, Vol. 22, pp. 76–108.

Ginter, R. L. 1930. "Causative Agents of Sulphate Reduction in Oil-Well Waters," *Bull. Amer. Assoc. Petrol. Geol.*, Vol. 14, pp. 139–52.

Goobin, V. M. 1923. "Assimilation of Hydrocarbons by Bacteria of the Tamboohanskand Petrovak Muds in Reference to Mud Formation," *Russian Health Resort Service*, Vol. 5, pp. 3–9.

Grant, C. W., and ZoBell, C. E. 1942. "Oxidation of Hydrocarbons by Marine Bacteria," *Proc. Soc. Exper. Biol. and Med.*, Vol. 51, pp. 266–67.

Gray, P. H. H., and Thornton, H. G. 1928. "Soil Bacteria That Decompose Certain Aromatic Compounds," *Centralbl. f. Bakt.*, Abt. II, Vol. 73, pp. 74–96.

Greig-Smith, R. 1914. "The Destruction of Paraffin by *Bacillus prodigiosus* and Soil Organisms," *Proc. Linn. Soc. N. S. Wales*, Vol. 39, pp. 538–41.

Haag, F. E. 1926. "Über die Bedeutung von Doppelbindungen im Paraffin des Handels für das Wachstum von Bakterien," *Arch. f. Hyg.*, Vol. 97, pp. 28–46.

———. 1927. "Die saprophytischen Mykobakterien," *Centralbl. f. Bakt.*, Abt. II, Vol. 71, pp. 1–45.

Haas, H. F. 1942. "The Bacterial Utilization of Petroleum Products and the Production of Carotinoid Pigments," thesis, Kansas State College, Manhattan. 94 pp.

Haas, H. F., Yantzi, M. F., and Bushnell, L. D. 1941. "Microbial Utilization of Hydrocarbons," *Trans. Kansas Acad. Sci.*, Vol. 44, pp. 39–45.

Harper, H. I. 1939. "The Effect of Natural Gas on the Growth of Microörganisms and the Accumulation of Nitrogen and Organic Matter in the Soil," *Soil Sci.*, Vol. 48, pp. 461–66.

Hessel, F. A. 1924. "Action des bacteries sur les hydrocarbures et en particulier sur les petroles," *Matieres Grasses*, Vol. 16, pp. 6936–40.

Hopkins, S. J., and Chibnall, A. C. 1932. "Growth of *Aspergillus versicolor* on Higher Paraffins," *Biochem. Jour.*, Vol. 26, pp. 133–42.

Johnson, F. H., Goodale, W. T., and Turkevich, J. 1942. "The Bacterial Oxidation of Hydrocarbons," *Jour. Cell. & Comp. Physiol.*, Vol. 19, pp. 163–72.

Kaserer, H. 1906. "Ueber die oxydation des Wasserstoffes und des Methans durch Microörganismen," *Centralbl. f. Bakt.*, Abt. II, Vol. 15, pp. 573–76.

Kegel, K. 1940. "Biological Reduction of Sulfates in Direct Trickling Gas Coolers," *Chem. Ztg.*, Vol. 64, p. 507.

Lantzsch, K. 1922. "*Actinomyces obligocarbophilus* (*Bacillus obligocarbophilus* Beij.), sein Formwachsel und seine Physiologie," *Centralbl. f. Bakt.*, Abt. II, Vol. 57, pp. 309–19.

Lipman, C. B., and Greenberg, L. 1932. "Petroleum Decomposing Organisms," *Nature*, Vol. 129, p. 204.

Maliyantz, A. A. 1935. "Bacteriological Method of Desulphurizing Crude," *Azerb. Neft. Khoz.* (U.S.S.R.), Vol. 6, pp. 89–93; abstract in *Jour. Inst. Petrol. Tech.*, Vol. 22, p. 772 (1936).

Matthews, A. 1924. "Partial Sterilization of Soil by Antiseptics," *Jour. Agric. Res.*, Vol. 14, pp. 1–57.

McCoy, A. W., and Keyte, W. R. 1934. "Present Interpretations of the Structural Theory for Oil and Gas Migration and Accumulation," *Problems of Petroleum Geology*, Amer. Assoc. Petrol. Geol., pp. 262–68.

Morrison, L. E., and Tanner, F. W. 1922. "Studies on Thermophilic Bacteria. I. Aerobic Thermophilic Bacteria from Water," *Jour. Bact.*, Vol. 7, pp. 343–66.

Münz, E. 1920. "Zur Physiologie der Methanbakterien," *Centralbl. f. Bakt.*, Abt. II, Vol. 51, p. 380.

Rahn, O. 1906. "Ein Paraffin zersetzender Schimmelpilz," *ibid.*, Vol. 16, pp. 382–84.

Rawn, A. M., Banta, A. P., and Pomeroy, R. 1939. "Multiple-Stage Sewage Sludge Digestion," *Trans. Amer. Soc. Civil Engin.*, Vol. 104, pp. 93–119.

Rittenberg, S. C. 1941. "Studies on Marine Sulphate-Reducing Bacteria," dissertation, University of California. 128 pp.

Sanderson, R. T. 1942. "Geomicrobiological prospecting," U. S. Patent Office, Patent No. 2,294,425, pp. 1–6.

Schollenberg, C. J. 1930. "Effect of Leaking Natural Gas upon Soil," *Soil Sci.*, Vol. 29, pp. 261–66.

Seyer, W. F. 1933. "Conversion of Fatty and Waxy Substances into Petroleum Hydrocarbons," *Bull. Amer. Assoc. Petrol. Geol.*, Vol. 17, pp. 1251–67.

Smith, J. E. 1931. "Venezuelan Oil-Field Waters," *ibid.*, Vol. 15, pp. 895–909.

Söhngen, N. L. 1906. "Ueber Bakterien welche Methan als Kohlenstoffnahrung und Energiequelle Gebrauchen," *Centralbl. f. Bakt.*, Abt. II, Vol. 15, pp. 513–17.

———. 1913a. "Benzin, Petroleum, Parafinöl, und Paraffin als Kohlenstoff- und Energiequelle für Mikroben," *ibid.*, Vol. 37, pp. 595–609.

———. 1913b. "Einfluss von Kolloiden auf mikrobiologische Prozesse," *ibid.*, Vol. 38, pp. 621–47.

Söhngen, N. L., and Fol, J. G. 1914. "Die Zersetzung des Kautschuks durch Mikroben," *ibid.*, Vol. 40, pp. 87–98.

Stone, R. W., Fenske, M. R., and White, A. G. C. 1942. "Bacteria Attacking Petroleum and Oil Fractions," *Jour. Bact.*, Vol. 44, pp. 169–77.

Störmer, K. 1908. "Über die Wirkung des Schwefelkohlenstoffs und ähnlicher Stoffe auf den Boden," *Centralbl. f. Bakt.*, Abt. II, Vol. 20, pp. 282–86.

Strawinski, R. J., and Stone, R. W. 1940. "The Utilization of Hydrocarbons by Bacteria," *Jour. Bact.*, Vol. 40, p. 461.

Sturm, L. D., and Orlova, S. I. 1937. "On the Transformation of Fat, Paraffin and Palmitic Acid under the Influence of Microörganisms from Ala-Kule-Lake," *Microbiology* (U.S.S.R.), Vol. 6, pp. 754–72.

Tauson, T. A. 1939. "Oxidation of Paraffin by Yeasts and Yeast-Like Organisms," *ibid.*, Vol. 8, pp. 828–32.

Tauson, V. O. 1928. "Bacterial Oxidation of Crude Oils," Neft. Yanoe. Khazyai Stvo., Vol. 14, pp. 220–30. Abstract in Chem. Abst., Vol. 23, p. 1431, 1929.

———. 1929. "The Oxidation of Benzene Hydrocarbons by Bacteria," *Planta*, Vol. 7, pp. 735–57.

Tauson, V. O., and Shapiro, S. L. 1934. "General Trend of the Oxidation of Oil by Bacteria," *Microbiology* (U.S.S.R.), Vol. 3, pp. 79–87; abstract in *Centralbl. f. Bakt.*, Abt. II, Vol. 92, pp. 287–88.

———. 1934b. "Allgemeine Richtung der Naphtha-oxydation durch Bakterien," *Centralbl. f. Bakt.*, Abt. II, Vol. 92, pp. 287–88.

Tausson, W. O. 1925. "Zur Frage ueber die Assimilation des Paraffins durch Mikroörganismen," *Biochem. Zeitschr.*, Vol. 155, pp. 356–68.

———. 1927. "Naphthalin als Kohlenstoffquelle für Bakterien," *Planta*, Vol. 4, pp. 214–56.

———. 1928. "Über die Oxydation der Wachse durch Mikroörganismen," *ibid.*, Vol. 193, pp. 85–93.

———. 1928b. "Die Oxydation des Phenanthrens durch Bakterien," *Planta*, Vol. 5, pp. 239–73.

Tausz, J. 1919. "Über die Einwirkung von Mikroörganismen auf Roherdöle," *Petrol. Zeitschr.*, Vol. 15, pp. 553–55.

Tausz, J., and Donath, P. 1930. "Über die Oxydation des Wasserstoffs und der Kohlenwasserstoffe mittels Bakterien," *Zeitschr. Physiol. Chem.*, Vol. 190, pp. 141–68.

Tausz, J., and Peter, N. 1919. "Neue Methode der Kohlenwasserstoff analyses mit Hilfe von Bakterien," *Centralbl. f. Bakt.*, Abt. II, Vol. 49, pp. 497–554.

Thayer, L. A. 1931. "Bacterial Genesis of Hydrocarbons from Fatty Acids," *Bull. Amer. Assoc. Petrol. Geol.*, Vol. 15, pp. 441–53.

———. 1936. "Present Status of the Hypothesis of Biogenesis of Petroleum," *A.I.M.E. Drilling and Production Practice*, Vol. 36, pp. 384–404.

Thaysen, A. C. 1939. "Gas Evolution in Gasoline-Storage Tanks Caused by the Activity of Microörganisms," *Jour. Inst. Petrol. Tech.*, Vol. 25, pp. 411–15.

Trask, P. D. 1932. *Origin and Environment of Source Sediments of Petroleum.* Gulf Publishing Company, Houston. 323 pp.

Umbreit, W. W. 1939. "Studies on the *Proactinomyces*," *Jour. Bact.*, Vol. 38, pp. 73–89.

Wagner, R. 1914. "Benzol Bacteria Which Can Utilize Benzol, Toluol, and Xylol as a Source of Carbon," *Zeitschr. f. Garungsphysiol.*, Vol. 4, pp. 289–319.

Wakengut, A. M. 1936. "A Hydrocarbon-Oxidizing Microörganism from Crude Oil," *Arch. Sci. Biol.*, Vol. 43, pp. 55–56.

ZoBell, C. E. 1938. "Studies on the Bacterial Flora of Marine Bottom Sediments," *Jour. Sed. Petrology*, Vol. 8, pp. 10–18.

———. 1940. "The Effect of Oxygen Tension on the Rate of Oxidation of Organic Matter in Sea Water by Bacteria," *Jour. Mar. Res.*, Vol. 3, pp. 211–23.

———. 1941. "Studies on Marine Bacteria. I. The Cultural Requirements of Heterotrophic Aerobes," *ibid.*, Vol. 4, pp. 42–75.

———. 1943. "Bacteria as Geological Agents with Particular Reference to Petroleum," *Petroleum World*, Vol. 40, pp. 30–43.

ZoBell, C. E., and Anderson, D. Q. 1936. "Vertical Distribution of Bacteria in Marine Sediments," *Bull. Amer. Assoc. Petrol. Geol.*, Vol. 20, pp. 258–69.

ZoBell, C. E., and Conn, J. E. 1940. "Studies on the Thermal Sensitivity of Marine Bacteria," *Jour. Bact.*, Vol. 40, pp. 223–38.

ZoBell, C. E., and Feltham, C. B. 1942. "The Bacterial Flora of a Marine Mud Flat as an Ecological Factor," *Ecol.*, Vol. 23, pp. 69–78.

ZoBell, C. E., and Grant, C. W. 1942. "The Bacterial Oxidation of Rubber," *Science*, Vol. 96, pp. 379–80.

Contribution No. 211

Reprinted from the JOURNAL OF MORPHOLOGY
Vol. 73, No. 2, September, 1943

STUDIES OF THE SUPRAVITALLY STAINED GOLGI APPARATUS

I. ITS CYCLE IN THE TECTIBRANCH MOLLUSC, NAVANAX INERMIS (COOPER)

LEONARD G. WORLEY AND ELIZABETH K. WORLEY
Department of Biology, Brooklyn College, Brooklyn, New York, and the Scripps Institution of Oceanography, La Jolla, California[1]

ONE TEXT FIGURE AND FIVE PLATES (TWENTY-NINE FIGURES)

Although most of our knowledge of the Golgi apparatus is based upon studies of fixed material there have been numerous attempts during the past two decades to elucidate its structure and behavior by means of vital dyes. The vital techniques, however, frequently present a picture at such variance with the conception established by the silver and osmic methods that they often are considered unreliable and, in general, they have not thus far succeeded at all in reconciling the various views and opinions concerning the Golgi apparatus. Some idea of the controversial state of the problem is furnished by the recent review of Kirkman and Severinghaus ('38) and after reading it one is inclined to agree with Dawson ('31) who says with regard to the identity of the apparatus that "the problem . . . appears to increase rather than decrease in complexity".

Much of this difficulty is clearly due to the fact that the apparatus exists in such a variety of forms in different cells and under different conditions in even the same cell. Accordingly, "it has been described as a fibrous reticulum, network, ring, or cylinder, a very irregular fenestrated plate, a more or less incomplete hollow sphere, vesicle, or cup, a collection of small spheres, rodlets and platelets or discs, a series of anastomosing canals, a group of vacuoles, and a differentiated region of homogeneous cytoplasm crossed by irregular interfaces'' (Kirkman and Severinghaus, '38). The relationship between these various forms is very incompletely understood. Indeed, several of these described differences are undoubtedly due to different interpretations of the same structure and to different degrees of osmication of the same figure. One of the most widely recognized characteristics of the Golgi apparatus is its supposed ''capricious'' behavior toward impregnation.

---

[1] Contribution from the Deparmtent of Biology, Brooklyn College, No. 44 and Contribution from the Scripps Institution of Oceanography, New Series No. 211.

An answer to some of these questions regarding the apparatus could be obtained if it were possible to follow it continuously over a considerable period of time in an intact group of cells, such cells not being removed from the organism. The developing eggs of some aquatic animals are remarkably satisfactory in this regard. This is particularly true of the eggs of marine molluscs where the Golgi apparatus, if certain precautions be taken, can be supravitally stained in the single-celled stage. Such eggs undergo typical cleavage and gastrulation, pass through the trochophore stage and become normal veligers several days later, the Golgi apparatus remaining stained throughout the period. It is the purpose of the present paper to describe our observations on such eggs and to discuss some of the controversial matters concerning the Golgi apparatus in the light of these observations.

The authors are indebted to various members of the Scripps Institution of Oceanography for assistance of various kinds and particularly to the Director of the laboratory and the Regents of the University of California for providing space and facilities for this work. We wish to thank Prof. Wesley R. Coe for checking some of our observations and for his critical reading of the manuscript.

MATERIAL AND METHODS

Eggs of the common Pacific tectibranch Navanax inermis (Cooper), were the chief objects of study, but reference also is made to material from the sea hare Tethys californica (Cooper), the bean clam Donax gouldii Dall, the sand tubeworm Sabellaria californica Fewkes and the nudibranchs Dendrodoris fulva (MacFarland) and, in particular, Hermissenda crassicornis (Esch.). Specimens of Navanax, known to be in a spawning condition, were brought from Mission Bay near La Jolla on February 5 and were kept in the laboratory in a large concrete aquarium supplied with running sea water. Every few days the water was drawn from the aquarium and the specimens allowed to lie uncovered for a few hours. This procedure seemed to stimulate egg laying, as eggs were always present on the morning following this operation. Eggs were usually deposited shortly after daybreak. The other species were kept in large battery jars supplied with running sea water. Tethys and Dendrodoris deposited eggs in October and November while Hermissenda spawned throughout the winter. Eggs of Sabellaria were secured in December by pulling individuals out of their tubes and allowing them to lie in a small quantity of water.

Embryologists have long been aware of the fact that when invertebrate eggs are placed into a very dilute solution of methylene blue, certain granules in the cells become intensely colored. In Navanax and Hermissenda, these granules first become red, then purple and finally, after 3 or 4 hours, dark blue. The form which these granules assume during cleavage leaves no doubt but that they are the embryonic Golgi elements and that the stain has colored the chromophilic (osmiophilic) component, while the chromophobic portion (osmiophobe) remains colorless. There is abundant proof at hand (see discussion) that the methylene blue does not alter the structure of these inclusions in any manner. In the healthy egg under the cover slip, a diffuse blue coloration of the cytoplasm quickly disappears as the methylene blue is reduced, but the stain is retained and gradually becomes more intense in the Golgi bodies.

The technique which we have found to work satisfactorily consists of immersing the eggs for about 45 minutes in very dilute methylene blue (Nat. Aniline Co.) of a concentration about 1 : 1,000,000, then placing them for a similar period in 1 : 750,000, later in 1 : 500,000 and so on, gradually over a period of 3 or 4 hours, bringing them into a 1 : 100,000 concentration. These operations should be carried out in very subdued light and the embryos subsequently kept in semi-darkness to avoid possible photodynamic effects (Tennant, '42). The methylene blue solutions are secured by diluting a concentrated stock solution made up in sea water several weeks in advance and subsequently allowed to ripen in the daylight. When properly employed, the methylene blue stains the Golgi elements of mollusc eggs dark blue, the mitochondria stain pale blue-green or not at all, while the fatty and albuminous yolk appear pale yellow-green. Child ('41) states with regard to "embryonic and larval stages of echinoderms" that toxic effects accompany staining with various vital dyes and that "toxic effect seems to depend on the accumulation of the dye on certain cell constituents which appear as deeply stained granules". In the concentrations we have employed, however, methylene blue is not toxic, but it is probable that carrying out these operations in semi-darkness and the gradual adaptation of the eggs to the stain eliminate toxic effects. Stained eggs develop normally and they keep pace with unstained controls developed at the same temperature. Both controls and stained embryos live the same length of time in the laboratory. An occasional egg or embryo may exhibit abnormalities which, however, are as likely to be due to mechanical injury as to the effect of the stain. Harvey ('41) has also found methylene blue to be "very innocuous" when used on the Arbacia egg. In Navanax and Hermissenda, when the technique is properly carried out, the ground cytoplasm and nucleus remain colorless after reduction. Whenever in our observations of eggs under the cover slip there has persisted, or later developed, a diffuse staining of either the cytoplasm or nucleus, the embryos have been considered abnormal and have been discarded. The appearance within the cytoplasm of violet droplets has also been considered a sign of injury.

Fixed preparations were used to compare with the living material and to aid in its interpretation. However, we soon found that we relied more and more upon the living material to interpret the structure of the fixed eggs rather than the reverse. As will be shown later, many of the appearances in fixed material find a ready explanation when compared with the living preparations. The following techniques for permanent slides were employed:

Regaud's fixative followed by crystal violet (Benda);

Regaud's fixative followed by aniline acid fuchsin and methyl green (Altmann);

Flemming's fixative with one drop of acetic per 5 cc. unstained;

Flemming's fixative with one drop of acetic per 5 cc. followed by Heidenhain's iron hematoxylin;

Mann-Kopsch;

Kolatchev-Nassonov.

THE FERTILIZED EGG

Eggs of Navanax are deposited after sperm entrance and the first polar body is formed shortly thereafter. A marked rearrangement of parts accompanies maturation, but we failed to secure stained eggs early enough to follow all of this process. Our earliest observations have been made on eggs in which the first polar body had been formed. At this stage, there appears to be a uniform distribution of both albuminous and fatty yolk throughout the egg except for a small area at the animal pole. The Golgi bodies are also widely scattered, but there are many small clusters of six to eight or more. No cortical differentiation of the egg was noted. The cytoplasm is loaded with albuminous yolk spheres which vary in diameter from 1.5 μ to about 6.5 μ. The lipoidal yolk is in the form of evenly distributed spheres of submicron size. Mitochondria, when seen, are wheat-grain shaped. They differ from the Golgi bodies in both shape and staining reaction and although in Kolatchev preparations these elements might be confused, they are clearly separable in vitally stained material. Little attention, however, has been paid to the mitochondria in this work.

Golgi bodies are abundant in the egg and, at this stage, are fairly uniform in size (under 1 μ to about 2 μ). Since the development of the Navanax egg proceeds very slowly, it is possible to get the Golgi elements sufficiently stained with methylene blue before the formation of the second polar body. In the freshly-laid egg they are in two forms, — homogeneous chromophilic granules of submicron size (fig. 1, a and fig. 6, A) and somewhat larger spheres showing a chromophilic "crescent" and a translucent chromophobic center (fig. 1, c and d and fig. 7). Golgi bodies of the second type resemble tiny signet rings, but are actually spherical or spherical with a chromophilic tubercle on one side (fig. 6, D). In osmicated material the chromophilic "crescent" or tubercle is black, while the remainder of the sphere is pale gray. As staining with methylene blue proceeds, the sphere which at first appears colorless or very pale greenish becomes very pale violet. This is due to the fact that the sphere (chromophobe) is surrounded by a thin pellicle which is a continuation of the chromophilic portion (chromophile). In a good Kolatchev preparation (fig. 14), this osmiophilic pellicle is therefore gray. If over-osmicated, it becomes black. The pellicle in life encloses a fluid of low viscosity which is actually chromophilic and probably osmiophilic, but which ordinarily is prevented from impregnation by the pellicle. When, however, the pellicle is ruptured so that the fluid escapes, the latter takes up the methylene blue stain rapidly. The sphere or vesicle is intensely fuchsinophilic after Regaud fixation and also shows a marked affinity for crystal violet and iron hematoxylin. It is at least as fuchsinophilic as the mitochondria and more so than the larger albuminous yolk spheres which also stain with fuchsin and crystal violet. For this reason, these spheres probably have occasionally been mistaken for mitochondria or for albuminous yolk and the yolk spheres have at times, because of this similarity in staining, been mistaken for mitochondria (in Limnaea, Gatenby, '19). The picture in fixed material is very confusing and becomes clear only when the eggs are vitally stained.

The small Golgi granules are osmiophilic, but they are also fuchsinophilic, stain with crystal violet and iron hematoxylin after Regaud fixation. The use of Regaud's fluid is particularly valuable as a fixative for these Golgi granules because they are not blackened by this technique and their staining reaction in comparison with that of the mitochondria can be observed. Because of their granular character and because, in Regaud-fixed eggs, they stain much like mitochondria, these Golgi bodies occasionally may have been mistaken for mitochondria in similar eggs. However, as already pointed out, the mitochondria in Navanax stain blue-green with mythelene blue, not navy blue and they are rod-shaped, not granular. Most important, many of these granular inclusions enlarge during maturation and cleavage and become Golgi spheres or vesicles. This was also noted in Aplysia limacina by Ries and Gersch ('36) who, however, called the Golgi bodies "Speichergranula" and were apparently unaware of their true identity. Other authors have observed the formation of "vesicles" "adjacent" to Golgi elements in vitally stained material, but have frequently attributed this to the action of the vital dye. The fact that such spheres arise in association with only some, not all, of the Golgi granules and because the sphere forms within, not "adjacent to" the granule, points strongly to the view that these spheres represent true Golgi developments, not "neoformations". Other authors have apparently considered that unenlarged granules in mollusc eggs were chondriosomes. This problem is further discussed later. The fact that Golgi bodies arise from tiny granules may have been one reason for Parat's ('28) theory that the Golgi bodies arose from chondriosomes ("chondriome actif").

Maturation is accompanied by a rearrangement of the parts of the egg, resulting in the accumulation of the albuminous yolk in the vegetal half and the fatty yolk in the animal hemisphere (fig. 2). The two regions thus stand in very sharp contrast as the egg is preparing for the first cleavage division. The Golgi bodies, unassociated with any of the other elements, occupy both parts of the egg but are clearly much more abundant in the animal portion. In both halves they are distributed indiscriminately. A few of the granular type may enter the polar bodies, particularly the first one. During this period, the small vesicular forms have been observed to exhibit rather remarkable changes in appearance and position. One sphere, for example, at 10:45 A.M. showed a chromophilic "crescent" extending about 150° around the near surface (6 o'clock to the eye). Within 1 minute, the center of the chromophile had shifted to about 3 o'clock, but by 10:47 A.M. was back at 6 o'clock; 20 seconds later it had moved to 4 o'clock and remained there until 10:49 A.M., but by 10:50 A.M. it was again at 3 o'clock and had extended 270° around the sphere. This rotation of the sphere might have been due to Brownian movement, but there was no other evidence of Brownian movement in the egg. Protoplasmic streaming, however, is very apparent in the single-celled stage and is very effective in shifting the inclusions about. The waxing, or waning, of the chromophile is a very common phenomenon and has been noted repeatedly. Sometimes a new "crescent" develops out of the pellicle on the side of the vesicle opposite the primary "crescent" (vertical arrow, fig. 12). As a consequence, the vesicle is enclosed within the concavities of the two "crescent"-like chromophiles (fig. 6, F). In many fixed preparations (fig. 23), these two chromophilic elements appear as parrallel rods due to the partial collapse of the chromophobe (see MacBride and Hewer, '31, p. 95). This bipolar charcater of the Golgi body is not, however, a prelude to division (compare Gatenby, '19). Furthermore, aside from the slight enlargement of the vesicle already referred to, these Golgi spheres have never been observed to be engaged in any synthetic process in the cell and we are inclined to regard them as more or less a "resting" type. The Golgi granules we consider to be "reserve" elements which are capable of enlargement into vesicles or more elaborate forms anytime the needs of the cell so dictate. There is a slight enlargement of the vesicular Golgi bodies during maturation and by the end of the period many of them are no longer spherical but have the chromophile in the form of a protrusion or tubercle on one side. The chromophile in these cases is shaped something like a bowl or like the base of a spherical light bulb (fig. 1, e and figs. 9 and 14).

One can keep an egg viable under a cover slip for hours if care is taken to avoid evaporation. However, when such precautions are not observed, the egg may rupture and its contents spread out on the slide. Under such circumstances, the Golgi bodies, contrary to what might be expected, show a remarkable degree of stability, remaining apparently unaltered for hours and drying up on the slide relatively undeformed. Attempts have been made by applying filter paper at the edge of preparations containing such isolated bodies to ascertain if differences in specific gravity exist between the chromophile and the chromophobe. Both these experiments and preliminary experiments in centrifuging the single-celled egg have given negative results on this point. Observations on isolated Golgi bodies do, however, strengthen the view that the vesicular type is radially symmetrical, the chromophile being more or less bowl shaped, but extending around the fluid chromophobe which it encloses.

The Golgi bodies of the fertilized egg are remarkably resistant to mechanical agitation, but are very sensitive to hydrostatic pressure. This is apparent when the egg, on desiccation, fails to collapse. In this case, the egg loses water as the surrounding fluid becomes more and more hypertonic and the egg undergoes a slight shrinkage. Under these circumstances, the Golgi vesicles rupture within the egg and their contents. almost immediately becoming chromophilic, are at once adsorbed on the adjacent albuminous or fatty yolk spheres if nearby; otherwise, they form "batonettes" or "dictyosomes" in the cytoplasm. This interpretation implies that a direct relation will exist between the size of the Golgi rims and the size of the yolk spheres. It is apparent in figures 10 and 11 that this is the case. The speed with which this adsorption takes place fully justifies the assertion that the content of the vesicle is a fluid at this stage in development. An albuminous yolk sphere together with the adsorbed Golgi material now bears a striking resemblance to a vesicular type Golgi body (compare figs. 12 horizontal arrow, and 13). Susceptible as the Golgi bodies are to slight shrinkage of the cell they presumably react in a comparable manner as a result of the slight shrinkage that accompanies osmic-without-acetic fixation. For this reason, osmiophilic crescents are often found surrounding albuminous yolk spheres in Kolatchev and Mann-Kopsch preparations of eggs, an association which in life does not exist but which is in part probably responsible for the idea held by some authors that the albuminous yolk arises under the influence of the Golgi bodies. The chance of making such an interpretation is even more likely, in an egg like that of Navanax, because every gradation in size exists between the smaller Golgi vesicles and the largest yolk spheres and since both Golgi vesicles and albuminous yolk spheres are fuchsinophilic in greater or lesser degree, the fixed picture does indeed give this impression. Figure 13 helps to make this point clear. In this photograph of a section through the vegetal hemisphere of a poorly fixed Dendrodoris egg, several of the large peripheral yolk spheres each have an osmiophilic rim facing the egg surface while those in the center of the egg lack these rims. The center of the egg, on the other hand, shows osmiophilic granules (Golgi bodes) which, however, are lacking toward the periphery. But in the living egg, these granules are evenly scattered throughout and it is apparent that in this specimen the fixative upon penetration has destroyed the more superficial Golgi bodies resulting in their contents being adsorbed by the albuminous yolk. Those in the interior have been fixed intact and as a consequence, the yolk remains unstained. The outer faces of the yolk spheres are blackened because the fixative on penetration has forced the Golgi body contents inward. Golgi vesicles rupturing and being adsorbed in a similar manner have been noted in vitally stained material every time we have studied an unbroken egg undergoing desiccation on the slide. These statements are not intended to mean that the albuminous yolk is not elaborated by the Golgi apparatus, but that the intimate association between the two in the fixed, fullgrown oöcyte and fertilized egg is an artifact. The Golgi vesicle in these stages in tectibranchs is in all probability neither fatty nor albuminous yolk in the ordinary sense.

Figure 14 is a photograph of one of the few fixed eggs in our collection which shows the vesicular Golgi bodies in a nearly unaltered condition. There is no close association here between the Golgi bodies and the yolk. The photograph is of the mature egg of Tethys and in this egg a grouping of the Golgi bodies around the nucleus occurs. Such an arrangement was not noted in Navanax in the single-celled egg, but does appear in the later cleavage stages. In Aplysia, a closely related species, Ries and Gersch ('36) found the Golgi bodies (''Speichergranula'') localized along the boundary between animal and vegetal cytoplasms. Such a localization in Navanax is delayed until the late four-celled stage (fig. 4).

Since there has been some contradictory evidence on differential dye reduction in these forms (Ries and Gersch, '36; Child, '41) it might be worthwhile to state here that in the mature eggs of Navanax, the polar bodies and animal polar region reduce the methylene blue rapidly and that the reduction progresses rather rapidly toward the vegetal pole, seeming to involve the surface first and later the interior of the egg.

CLEAVAGE AND EARLY GASTRULATION

The first cleavage results in a passive distribution of the Golgi bodies to the two daughter cells (fig. 7). There is no evidence whatever of any form of ''dictyokinesis'' at this stage nor at any time during cleavage and gastrulation. The total number of Golgi bodies is believed to remain constant or nearly so until the veliger stage, but due to the slow enlargement of the Golgi bodies that is taking place, their total volume is certainly increasing and because of this increase in size they sometimes give the impression of being more numerous. There is, following the first cleavage, a migration of the albuminous yolk to the more lateral regions of the embryo so that this yolk becomes both vegetal and lateral in position while the fatty yolk and most of the cytoplasm is more central. The albuminous yolk is now distributed in the egg somewhat in the form of a thick walled cup, while the animal material occupies the lumen of this cup. A movement of some of the Golgi bodies into the albuminous yolk region has been noted during this period.

The 2-celled stage is attained in Navanax (at 20°C.) about 5 hours after laying; the 4-celled condition about 1½ hours later. The second cleavage produces four cells which appear to be cytologically equivalent (fig. 3). Preceeding the third cleavage, a migration of all of the Golgi bodies to the site of the third furrow occurs (fig. 4). There is thus formed, in stained eggs, a dark blue supra-equatorial band. The furrow splits this band longitudinally with the result that there is what appears to be a nearly equal allocation of the Golgi elements to each of the eight daughter cells (fig. 5). It is apparent that had no such localization taken place, the third cleavage would have isolated the vast majority of the Golgi bodies in the animal cells. The micromeres receive no albuminous yolk and are, of course, smaller than the macromeres partly for this reason; otherwise all eight cells appear to be cytologically equivalent. During the 8-celled stage, a slight enlargement of the Golgi bodies has been noted, but there is no change in their structure.

The fourth cleavage, which in Navanax (at 20°C.) occurs about 8 hours after laying, produces twelve cells, eight of which (the micromeres) are composed of ground cytoplasm and fatty yolk while the four macromeres have albuminous yolk in addition. At this time and during the subsequent stages of the cleavage period, there is no appreciable change in the Golgi bodies (fig. 9), nor is there any apparent activity during the greater part of gastrulation. Ectoderm and endoderm cells show no cytological differences except in the amount and character of the yolk present. There is throughout the cleavage and gastrulation stages, however, a steady diminution in the amount of albuminous and fatty yolk. By the close of gastrulation the amount of albuminous yolk appears to have been reduced to about half that of the fertilized egg. Meyerhof ('11) concluded from his studies of the calorific quotients in the developing egg of Aplysia that ''fat was being burned as a source of energy throughout development''. This is borne out by microscopic inspection. No quantitative data are at hand regarding this point, but it is obvious that the number of fatty yolk droplets has greatly diminished between the one-celled stage and the late gastrula.

SYNTHESIS IN THE TROCHOPHORE

Beginning at about the end of gastrulation (30 hours of development at 20°C.) as the embryo is preparing to transform into a trochophore, the Golgi bodies begin to renew both the fatty and albuminous yolk content of the egg and in doing so they become much elaborated. This burst of activity continues through the trochophore and subsides in the early veliger. It is interesting to note that in Aplysia, Buglia ('08) demonstrated a considerable increase in the respiratory metabolism of the embryo at just this same period, followed by a decrease in the early veliger. This coincidence between Golgi body activity and respiratory metabolism is probably not accidental.

As stated earlier, fatty yolk droplets are much smaller than albuminous yolk spheres. The fatty yolk droplets are probably not more than a cubic micron in volume, while the largest albuminous yolk spheres probably attain a volume near 100 μ^3. Perhaps as a consequence of this fact, the albuminous yolk spheres take much longer to develop; i.e., about 55 or 60 hours, while a fatty yolk droplet can be elaborated and discharged within an hour. There is, therefore, a rough relation between the size of the yolk droplet, whether fatty or albuminous, and the period required for its elaboration. Although albuminous yolk synthesis has obviously started by the close of gastrulation (fig. 12), the discharge of the mature product has never been observed until the early veliger 2 days later. A single Golgi body may, however, elaborate two or three protein yolk spheres simultaneously (fig. 6, H). What is even more remarkable, the elaboration of both fatty and albuminous yolk may take place simultaneously within the same Golgi body (fig. 6, J). Many of the Golgi bodies engage solely in the synthesis of either one or the other, but there are fully as many that carry on both activities. The albuminous yolk is elaborated through the enlargement of the original chromophobic vesicle. During this process the pellicle remains thin and the yolk can be seen inside. Within the thicker portions of the chromophile, most of the fatty yolk droplets are elaborated, but occasionally they also make their appearance in the pellicular wall.

Those Golgi elements that engage solely in the synthesis of fat have thick walls and they resemble a mass of puddingstone in which the chromophile forms the continuous phase and the fat droplets constitute the interrupted phase (figs. 6, I, 25 and 26). Golgi bodies engaged solely in the synthesis of albuminous yolk, however, resemble a small piece of foam, the chromophile constituting the walls and the yolk occupying the spaces within. In such masses, the chromophile is always thicker in the center or centrally and thinner in the pellicular portions. Those Golgi bodies that are simultaneously engaged in the synthesis of both protein and fat (fig. 1, h, i and j and fig. 6, J) typically develop the fat out of the central areas, the albuminous spheres being more peripheral. In such a case, a bipolar Golgi body may be formed having central fat and terminal protein. Unipolar bodies, however, are more common; in these cases, the fat occupies the thicker portion of the chromophile at one end and the protein spheres the opposite. Elaboration and discharge of fat progresses rapidly during the trochophore stage and is occurring simultaneously throughout the body, but is particularly noticeable in the prototroch because there the Golgi elements are more abundant and they are much more numerous in the animal cells than in the vegetal. New vesicles, singly or in pairs, are constantly appearing within the chromophile and upon reaching a certain size may be discharged as fat or they may continue to enlarge into protein droplets to be cast out much later. When they first appear, it is impossible to determine whether they will become fat or protein, but those that develop beyond about 1 μ are discharged in the veliger as solid albuminous yolk bodies. The Golgi bodies when first showing enlargement may lie close to the nuclear membrane, but they enlarge without orientation. Most of the Golgi elements of the embryo are involved in this synthetic process to a greater or lesser degree so that only a minority of ''reserve'' granules is left. During development, an apparent waxing and waning of the chromophile has been noted, but it could not positively be determined that this was not due to a shifting in position of the Golgi body under observation. As many as eighteen or twenty fat droplets have been observed in some Golgi bodies and, at the height of activity, these may be discharged, at least as frequently as one every 5 minutes. That these small droplets are fat is indicated by their rapid (1 to 2 minutes) browning when a small drop of 2% osmic acid is placed on the material. The larger yolk spheres do not turn brown. Fat synthesis is characteristic of the trochophore stage, beginning about 30 hours after fertilization and continuing until late in the third or beginning of the fourth day when it subsides and the embryo metamorphoses into the veliger.

Much information regarding the nature of these hypertrophied Golgi bodies can be gained by observing the effect of desiccation upon them (figs. 15–22). If freed from the egg by rupture of the cytoplasm, they flow unaltered onto the slide and retain their original shape for a long period. If, however, due to shrinkage of the cell through evaporation, they are caused to collapse, they gradually take on a much modified appearance. Figures 15–18 show 4 stages in such desiccation. The content of the vesicles is now much less chromophilic and becomes on discharge pale reddish-violet, rather than navy blue as in the earlier period. It is also now much more viscous than in the cleavage stages and upon collapse fragments of it remain attached to the chromophile. Desiccated Golgi bodies consequently appear as chromophilic ''dictyosomes'', loops, rods or ''batonettes'' and even as simple networks to which are attached remnants of the chromophobe, the former vesicles (figs. 19–22). It is for this reason that the chromophobe occurs on only one side of the chromophile in the fixed egg (see MacBride and Hewer, '31, p. 96). The artificial picture thus obtained bears so little resemblance to the original that one could not possibly appreciate or understand the original by studying only these artifacts. One is tempted to suggest that there have been occasions in other material when the fixed picture resembled the original no more closely (compare figs. 19–22 with Hirschler's ('18) drawings of the fixed Golgi bodies in Limnaea).

SYNTHESIS AND DIVISION IN THE VELIGER

By the early veliger stage (fourth day) the synthesis of fatty yolk has largely ceased. The chromophiles, still quite elaborate at the beginning of this period, eventually begin to withdraw from the albuminous yolk spheres which, up to this time, they have enclosed (fig. 29, arrows pointing downward). In this process the chromophile shrinks up into an irregular, homogeneous, chromophilic mass showing no internal structure (fig. 1, k and figs. 27 and 29). As a result of this pycnosis, the protein sphere is released and it emerges completely free of any chromophilic matter. This is the first time within the history of the embryo that the animal cells have contained albuminous yolk as formed bodies, although they have been endowed from the beginning with numerous Golgi bodies each containing a vesicle of potential albuminous matter. The Golgi vesicles are thought not to have contained albuminous yolk in the ordinary sense since the staining reaction of the two is much different in degree though alike in kind. Likewise, the content of the vesicle began as a fluid in the single-celled stage, becomes semi-fluid in the trochophore and finally emerges in the veliger as a more or less solid body.

Pycnosis of the Golgi bodies is a transitory condition, the first step in Golgi body multiplication. The Golgi bodies have shown marked activity during the trochophore, but they have never been observed to divide. Now, however, without any relation to cell division, the pycnotic Golgi body breaks up into droplets which move slowly apart and eventually become well scattered in the cytoplasm (fig. 1, l and figs. 28 and 29). This process follows so closely on the heels of albuminous yolk discharge that frequently the one is not over before the other begins. Thus the pellicle may resolve itself into droplets and release the protein sphere in that manner (fig. 29, diagonal arrow). These Golgi droplets are of a uniform size and the number that emerge from the condensed Golgi element depends on the volume of the pycnotic mass. The number thus varies from four or five to perhaps as many as twenty. Any one of these droplets resembles an original Golgi granule in its primary state of enlargement and as these are watched during the next few hours they are seen to develop pale vesicular centers. In general, during the next 72 hours the Golgi bodies remain more or less inactive although there are local areas of activity. Most of the Golgi bodies lie passively in the cytoplasm and can probably be considered ''resting'' forms. In both structure and lack of activity they are like the ''resting'' forms of the early cleavage stages (compare Golgi body at diagonal arrow, fig. 29 with those at arrows, fig. 7). That these elements do undergo elaboration later is apparent from an examination of the Golgi apparatus in adult tissues (in Donax) but there is good reason to believe that the various forms shown by the Golgi apparatus in the different adult cells have been attained through the same steps in elaboration as shown in the trochophore and veliger.

DISCUSSION

In Navanax, therefore, and no doubt in other species, the Golgi apparatus exhibits a regular cycle in its morphological changes and its physiological activities (fig. 1). That it passes through this cycle more than once during the life history of the

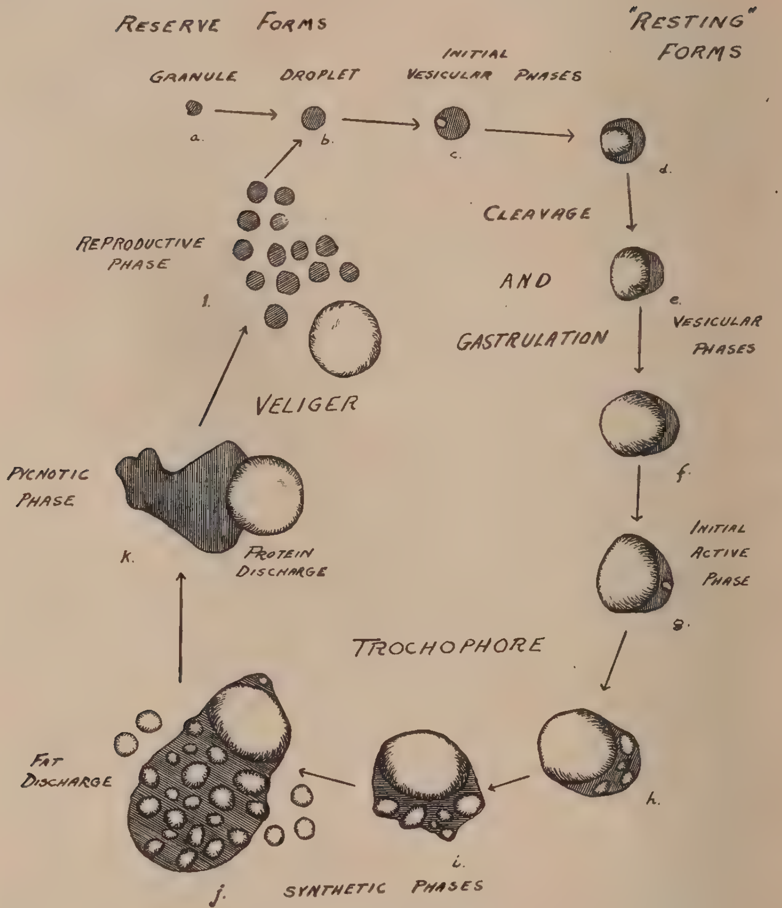

RESERVE FORMS
"RESTING" FORMS
GRANULE
DROPLET
INITIAL VESICULAR PHASES
a.
b.
c.
d.
CLEAVAGE AND GASTRULATION
e.
VESICULAR PHASES
f.
INITIAL ACTIVE PHASE
g.
TROCHOPHORE
h.
i.
SYNTHETIC PHASES
j.
FAT DISCHARGE
PYCNOTIC PHASE
k.
PROTEIN DISCHARGE
VELIGER
REPRODUCTIVE PHASE
l.

Fig. 1 Diagram of the cycle of the Golgi apparatus in the early development of Navanax. Granules and droplets (a and b) of the fertilized egg are interpreted as reserve forms which through enlargement and internal differentiation develop into the vesicular phases (c, d and e) during cleavage and early gastrulation. These simple vesicular types are considered inactive or ''resting'' forms which through further enlargement and the development of new vesicles (f, g, h, i and j) become the active forms of the trochophore. These types are engaged in the synthesis of albuminous yolk and fat, the latter being discharged throughout the trochophore stage while the albuminous yolk is carried into the early veliger. Through pycnosis of the Golgi apparatus (k) the albuminous yolk is discharged and, shortly after, the pycnotic body breaks up into droplets (l) of the same size and appearance as the original droplet (b). Many of these droplets soon enter the initial vesicular phases (c and d), but the majority show no further development for at least several days.

organism is at once apparent. An examination of supravitally stained young oöcytes of the ovary (in Mytilus and Donax) reveals the Golgi apparatus in the active secretory condition, presumably synthesizing yolk (fig. 8). In the freshly laid egg of Navanax, however, these synthetic forms are no longer present, the apparatus having passed through pycnosis and into division before laying. It is probably for this reason that clusters of Golgi bodies are found in the cytoplasm of the single-celled stage; division has occurred, but the daughter elements have not yet become completely scattered. Examination of the adult cells of Donax reveals the Golgi bodies in a variety of forms all, however, conforming to one of the steps in the Navanax cycle. There are droplet forms, pycnotic forms and simple vesicular forms clearly indicating that following the reproductive phase in the larva, some of the elements have undergone differentiation which has been more or less suspended as the organism has attained its fully differentiated form. Perhaps many of these elements in the adult serve no function, but this is, of course, not true in such organs as certain glands, etc. where the apparatus probably remains a tive throughout life. Hypertrophy of the apparatus has, therefore, occurred at least once during oögenesis, again in the trochophore and finally again in the differentiation of the gland cells.

During the Golgi cycle, the same elements have existed as granules, droplets, relatively large spheres or vesicles, as a collection of small spheres or vesicles, as a puddingstone-like reticulum enclosing fat droplets and protein and as an irregular homogenous mass, all within the space of a few days. The apparatus has not existed in the living condition as a ring or as a cylinder, nor as a fenestrated plate, nor as a cup, rodlet, platelet or disc, but it has been seen in all of these forms in desiccated material. We have thus, in Navanax, encountered most of the described forms of the apparatus, but it is probable that other forms of the apparatus do exist in the living condition in other species. It seems appropriate to suggest that some of the figures in the literature which have been interpreted as typical Golgi bodies in molluscs have, in actuality, been artificial forms which have been produced by slight shrinkage during fixation. At the same time, some of the osmic preparations heretofor considered worthless because the cells contained only a group of blackened droplets may possibly have presented fairly faithfully the real Golgi material. This has certainly been our experience with Navanax, Tethys, Donax and Hermissenda. It is interesting to note in this connection that Pollister ('41) has stated that with the notable exception of the egg cell, mitochondria are probably always filamentous or rod-like, never granular. Our experience with egg cells of molluscs leads us to believe that this assertion can be extended to include them also, the chromophilic granular inclusions in the egg being Golgi bodies, not mitochondria.

When we first began to notice the elaborate structures into which the Golgi bodies developed, we were much concerned that such development might be due to the effect of the vital stain on the material. Some vital dyes have a reputation for bringing about the formation of artificial structures in the cell but, according to von Möllendorf ('20), although acid dyes are segregated in cells and produce new formations (vacuoles and granules) basic dyes differ in that they stain only preformed structures. The basic dye methylene blue, we have noticed, does form violet droplets of large size in injured or dying cells, but no such structures appear in the normal egg. We have thoroughly explored the possibility that the elaborate Golgi bodies we have seen are "neoformations" and find no ground for believing that they are and abundant evidence that they are not. This evidence may be summarized as follows:

1. Controls and stained animals develop at the same rate into perfectly normal veligers all of the same form, size and behavior, and both controls and stained individuals live equally long under laboratory conditions, all dying at about the same time in the same state of development.

2. These staining experiments have been repeated on twenty different batches of Navanax eggs at different times during

the spring and on twelve different batches of Hermissenda eggs. The behavior of the Golgi bodies has always been identical. For example, in the apical region of the Hermissenda trochophore two, and only two, particularly large Golgi bodies always appear in the same position and at the same time.

3. When an unstained trochophore is crushed on the slide so that its contents flow out and can be examined, Golgi bodies as numerous and as elaborate as those in the stained cell are found. The chromophile has a slightly higher refractive index than the chromophobe and the two can be distinguished.

4. Golgi bodies of the type described are found in a somewhat shrunken and distorted form in osmicated sections of oöcytes and embryos.

5. The degree of elaboration is not dependent on the concentration of the stain. The bodies are of the same form whether cultured in 1:500,000 or 1:100,000 methylene blue.

6. When unstained late gastrulae or trochophores are placed in methylene blue, elaborate Golgi bodies are visible within 2 hours, but when single-celled stages are treated in exactly the same manner, elaborate Golgi bodies do not appear for 30 hours, i.e., not until they reach the late gastrula stage.

7. If methylene blue were responsible for the observed developments one would expect it to affect all of the Golgi elements similarly, yet many of them develop into vesicles while many others are left behind as reserve granules which lie side by side with the more elaborate types.

8. The Golgi bodies which occasionally enter the polar bodies never enlarge although they can be followed for a day or more.

9. The chromophobic vesicles always develop within the chromophile, never on the surface.

10. A pycnosis of the Golgi elements occurs in the veliger which is the opposite of the expected effect if methylene blue is considered capable of producing ''neoformations''. This pycnosis cannot be affected by increasing the methylene blue concentration or by placing the embryos in unstained water.

11. The picture presented by desiccated Golgi bodies so closely resembles what is undoubtedly the Golgi material in the fixed and osmicated cells, that it seems certain they are identical.

12. Finally, the activity shown by these elaborate Golgi bodies is in such harmony with all of the known facts about the apparatus, and their behavior in the elaboration of protein and fat is so obviously of value in the economy of the organism that the possibility that the general facts outlined above have been misinterpreted does not seem very likely.

Activity of the Golgi bodies of the sort described has not previously been observed because no one has studied these inclusions in the appropriate developmental stages. It is remarkable that there are so few published accounts of the Golgi apparatus in the developing embryo. Gatenby ('19) followed the Golgi apparatus through oögenesis and into the gastrula in the snail (Limnaea) and concluded that this inclusion could be identified in the embryonic stages. Hirschler ('18) previously had made a much more complete study (in Limnaea), but seems to have skipped the very stages which we have found to be of greatest interest. His figures of the Golgi apparatus in cleavage stages resemble our vesicular forms and he found an increase in their size in the gastrula as did we. His ''Larvenstadium'' appears to be an older embryo than the Navanax early veliger. Hirschler describes the mitochondria in the cleavage stages as granular, a statement with which we do not agree, for reasons already given. In this, our observations coincide with those of Ries and Gersch ('36). Hirschler's figures of the mitochondria in later stages, however, picture them much as they have been observed in Navanax. Parat ('28) figures cells of the blastula and planula of Chrysaora in which what are probably the Golgi bodies are stained with neutral red, but the neutral red bodies in his figure of the advanced gastrula of Aplysia bear little or no resemblance to the Golgi bodies demonstrated with methylene blue in Navanax at a similar stage. It appears probable, that in some cases, the ''vacuome'' may be synonomous with our chromophobe. However, as pointed out above, droplets unrelated to the Golgi apparatus sometimes do develop in unhealthy cells vitally stained. Whether or not these are considered ''vacuome'', they are certainly not the chromophobe. Nevertheless, since the Golgi apparatus does at times exist in the form of droplets and since these droplets can be supravitally stained with methylene blue, they may also be capable of taking up neutral red and in such cases coincide with the ''vacuome''. On the other hand, Parat's early suggestion that the Golgi apparatus is an osmic or silver precipitation on the surface of the vacuome is totally erroneous. Likewise, any feeling that may still exist that the Golgi apparatus is a cellular artifact is completely outdated.

SUMMARY

1. The Golgi apparatus, supravitally stained with methylene blue, has been followed continuously in the developing eggs of Navanax from the single-celled stage through the veliger larva. The methylene blue technique stains the chromophilic portion of the apparatus navy blue, but leaves the chromophobic portion unstained or only slightly stained. Mitochondria are stained pale blue-green or not at all.

2. During cleavage and early gastrulation, the apparatus is in the form of tiny chromophilic granules or small spherical vesicles with pale centers and a chromophilic pellicle, thickened on one or more surfaces as a tubercle or bowl-shaped ''crescent''. The granule is interpreted as the most simple and undifferentiated form of the apparatus and is thought to be a ''reserve'' type. Granular Golgi bodies enlarge and become simple vesicles which are considered inactive or ''resting'' forms.

3. During late gastrulation, many of the small vesicles undergo considerable enlargement and in the early trochophore become very elaborate, as many as eighteen or twenty new vesicles developing out of the chromophile of each hypertrophied Golgi body. Those vesicles which attain a diameter of about 1 μ may be discharged from the apparatus in the form

of minute fat droplets in the trochophore. Or they may continue to enlarge and be carried until the early veliger when they are discharged as protein yolk spheres (2–6 μ in diameter). Fat discharge is characteristic of the trochophore; protein yolk discharge is characteristic of the early veliger. Both fat and protein may be elaborated simultaneously within the same Golgi body; or two or three protein yolk spheres may be elaborated simultaneously. Fat droplets can be elaborated by the Golgi apparatus in less than an hour; the largest protein spheres require 2 days or more.

4. The discharge of the protein yolk in the early veliger is coincident with a pycnosis of the Golgi apparatus, which thus becomes a homogeneous, irregular chromophilic mass. The breaking up of this mass into numerous Golgi droplets follows, the number in direct proportion to the volume of the pycnotic mass since all of the droplets are of the same size. This is the only stage during the first 8 days of development when the Golgi elements are believed to multiply. Their division has no relation to cytoplasmic division. The daughter Golgi elements thus formed are of the same size and character as those of cleavage stages and show no activity during the succeeding 3 days.

5. It is concluded that the apparatus exhibits a regular cycle of activity and multiplication and that both fat and protein yolk are elaborated through the direct action of the Golgi apparatus.

6. All of the mitochondria of the egg are rod-shaped, all chromophilic granules being interpreted as Golgi elements. The staining reaction of mitochondria and Golgi apparatus to acid fuchsin, crystal violet and iron hematoxylin is similar, but to methylene blue is much different.

7. It is concluded that in life the Golgi apparatus in molluscs exists in the following forms: granule, droplet, sphere or vesicle with chromophilic surface, puddingstone-like reticulum enclosing fat or protein or both, pycnotic mass. Rings, cylinders, fenestrated plates, cups, rodlets, platelets and discs are considered artifacts in the mollusc, as they are never seen

in life, but appear only in fixed material or eggs and embryos that have undergone desiccation on the slide.

8. All forms of the appartus show remarkable ability to maintain their shape when they are isolated from the egg, but when subjected to the slightest hydrostatic pressure by shrinkage, they quickly collapse and become much deformed.

9. The contents of the Golgi vesicle is a fluid of low viscosity in the cleavage stages, is semi-solid in the early trochophore and is more or less solid when discharged as fat or protein.

LITERATURE CITED

BUGLIA, G. 1908 Arch. di Fisiol., vol. 5, p. 455. (Cited from Needham, 1931).

CHILD, C. M. 1941 Patterns and Problems of Development. University of Chicago Press.

DAWSON, A. B. 1931 The 'zone of Golgi' in the cartilage cells of Necturus. Anat. Rec., vol. 48, pp. 379–398.

GATENBY, J. B. 1919 V. The gametogenesis and early development of Limnaea stagnalis (L.), with special reference to the Golgi apparatus and mitochondria. Quart. J. Micr. Sci., vol. 63, pp. 445–492.

HARVEY, E. B. 1941 Vital staining of the centrifuged Arbacia punctulata egg. Biol. Bull., vol. 81, pp. 114–118.

HIRSCHLER, J. 1918 Ueber den Golgischen Apparat embryonaler Zellen. Untersuchungen an Embryonen von Limnaeus stagnalis L. Mollusca. Arch. f. Mik. Anat., Bd. 91, S. 140–181.

KIRKMAN, H., AND A. E. SEVERINGHAUS 1938 A review of the Golgi apparatus. Anat. Rec., vol. 70, pp. 413–432; 557–573 and vol. 71, pp. 79–103.

LUDFORD, R. J. 1930 III. Vital staining of acinar cells of the pancreas and its bearing on the theories of vital staining with basic dyes. Proc. Roy. Soc. London, B., vol. 107, pp. 101–114.

MACBRIDE, E. W., AND H. R. HEWER 1931 In: Recent Advances in Microscopy. Churchill, London.

MEYERHOF, O. 1911 Biochem. Zeitschr., Bd. 35, S. 316. (Quoted from Needham, 1931).

VON MÖLLENDORF, W. 1920 Ergeb. Physiol., Bd. 18, S. 151. (Cited from Ludford, 1930).

NEEDHAM, J. 1931 Chemical Embryology. University Press, Cambridge.

PARAT, M. 1928 Contribution a l'étude morphologique et physiologique du cytoplasme; Chondriome, vacuome (appareil de Golgi), énclaves, etc. . . . Arch. d'Mik. Anat., T. 24, pp. 73–357.

POLLISTER, A. W. 1941 Mitochondrial orientations and molecular patterns. Physiol. Zoöl., vol. 14, pp. 268–280.

RIES, E., AND M. GERSCH 1936 Die Zelldifferenzierung und Zellspezialisierung während der Embryonalentwicklung von Aplysia limacina L. zugleich ein Beitrag zu Problemen der vitalen Färbung. Pub. de Sta. Zool. Napoli, vol. 15, pp. 223–273.

TENNANT, D. H. 1942 The photodynamic action of dyes on the eggs of the sea urchin, Lytechinus variegatus. Carnegie Inst. Wash. Pub. 539; Papers Tortugas Lab., vol. 35, pp. 1–153.

PLATE 1

EXPLANATION OF FIGURES

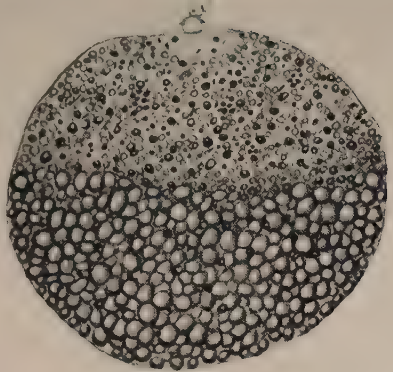
All stated magnifications are approximate.

2 Camera lucida drawing (side view × 420) of the mature, fertilized Navanax egg showing the localization of the large albuminous yolk spheres in the vegetal half and the small (pale) fatty yolk spheres in the animal hemisphere. Golgi bodies (black granules and lop-sided vesicles) are distributed throughout, but are more abundant in the animal portion. One small Golgi granule has entered one of the polar bodies and others are scattered in the yolk-free area near the animal pole. Rod-shaped mitochondria are scattered in the endoplasm.

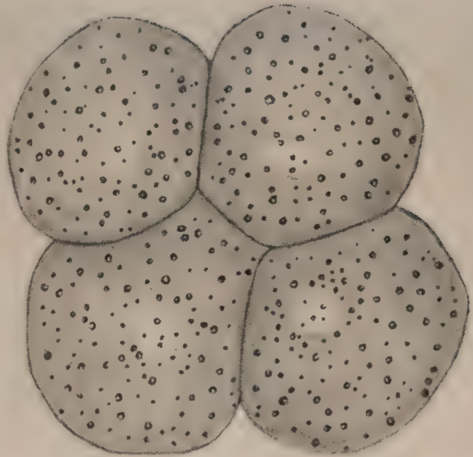
3 Polar view of 4-celled stage soon after the second cleavage showing the even distribution of the Golgi bodies in the animal cytoplasm. Yolk and mitochondria have been omitted. Compare with figure 4. Camera lucida × 420.

4 Localization of the Golgi bodies along the future third cleavage furrow in the late 4-celled stage. As a consequence, there is a nearly equal distribution of these elements to the eight daughter cells. Mitochondria have been omitted. Camera lucida × 420.

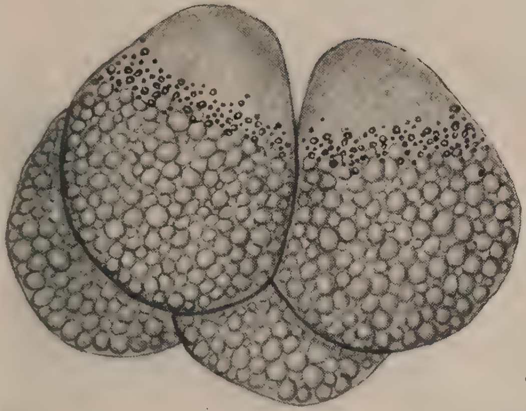
5 Eight-celled stage (side view × 420). The Golgi bodies are again evenly distributed in the animal cytoplasm and they have undergone a slight enlargement since the single-celled stage. Those in the polar body have not enlarged. Fatty yolk has been omitted.



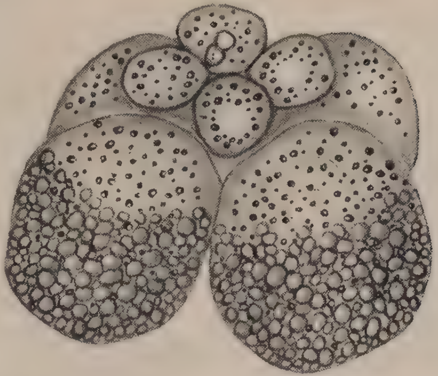
2



3



4



5

PLATE 2

EXPLANATION OF FIGURE

6 Living Golgi bodies of the prototroch of the Navanax trochophore (× 2000), supravitally stained with methylene blue. The chromophile (osmiophile) appears black; the chromophobe (osmiophobe) appears very pale gray. A, ''reserve'' granule; B, droplet; C, initial vesicular phase (others in fig. 7); D, E and F, ''resting'' or inactive vesicular forms; D, tuberculate type; E, typical cup type; F, bipolar cup type; G, initial active phase; H, I and J, synthetic phases; H, type chiefly albuminous yolk forming; I, type chiefly fat forming; J, type engaged in the synthesis of both fatty and albuminous yolk. Pycnotic phases may be seen in figures 27 and 29; reproductive phases in figures 27, 28 and 29.

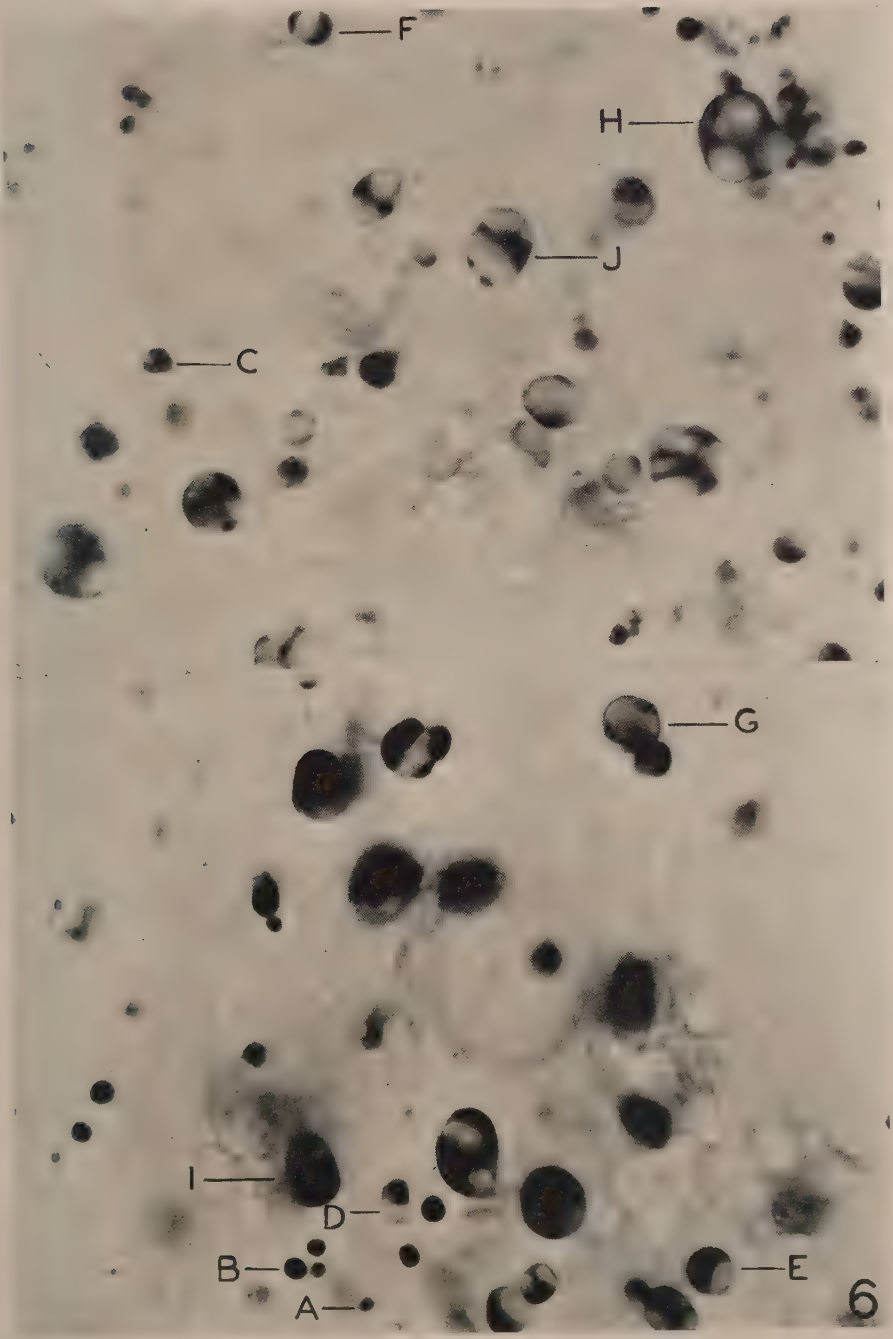

F
H
J
C
G
I
D
B
E
A
6

PLATE 3

EXPLANATION OF FIGURES

7 Two-celled stage of Hermissenda (× 1000) seen through the egg capsule. Arrows point to Golgi bodies of early vesicular phases in side view. Other Golgi bodies, both granular and vesicular forms, are scattered in the cytoplasm. Vitally stained with methylene blue and somewhat flattened under the cover slip.

8 Somewhat over-osmicated section (× 1200) of an ovarian egg of Donax showing shrunken and distorted Golgi inclusions of the active types and Golgi granules in the cytoplasm.

9 Sixteen-celled stage of Hermissenda (× 1000) showing character of Golgi inclusions. Vitally stained and viewed through the egg capsule. Golgi bodies of the vesicular type somewhat larger than those of figure 7 are indicated by arrows.

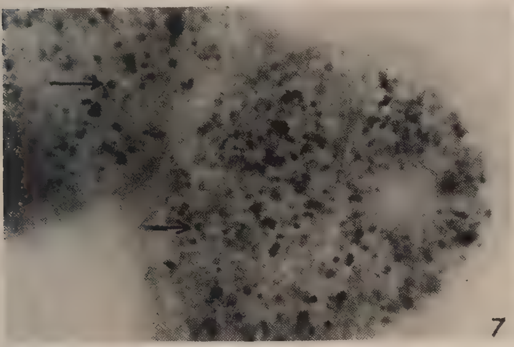
10 View of animal cytoplasm of 2-celled Hermissenda egg (× 1000) vitally stained and then subjected to about 2 hours desiccation on the slide. Golgi artifacts in the form of crescents, rings, and other dictyosomes have resulted from slight desiccation shrinkage. The size of the crescents is in direct proportion to the size of the fatty yolk spheres. Compare with figure 11.

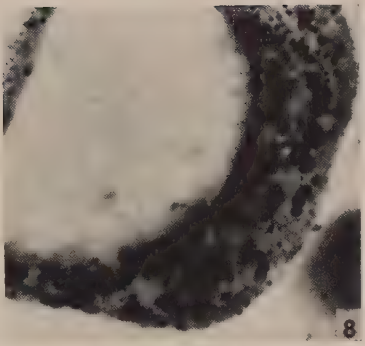
11 View of vegetal cytoplasm of 4-celled Hermissenda egg (× 1000), vitally stained and then subjected to about 2 hours desiccation on the slide. Golgi artifacts, crescents, rings and rods have developed as a result of shrinkage. Many of the crescents are larger here than in figure 10, because they are associated with large albuminous yolk spheres.

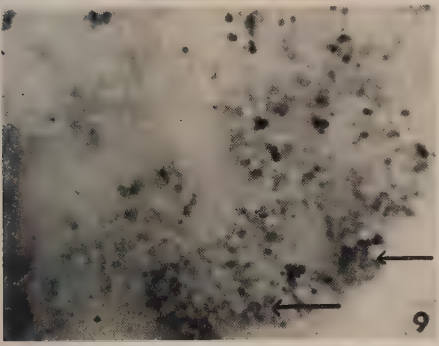
12 View through the capsule of the late gastrula of Hermissenda (× 1000) about 30 hours after fertilization, showing the enlargement of the Golgi bodies preceding the active phases of the trochophore. The vertical arrow indicates a Golgi body of the bipolar simple vesicular type. Vital methylene blue staining.

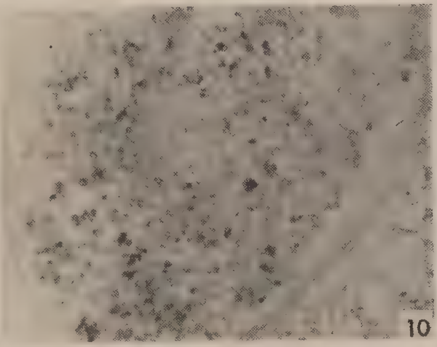
13 Poorly fixed single-celled egg of Dendrodoris (× 1000) showing artificial Golgi rims on albuminous yolk spheres. Note the similarity between these artifacts and the living Golgi bodies of the late gastrula (fig. 12, horizontal arrow). In the center of the field unruptured Golgi granules are visible. Mann-Kopsch technique, 4 μ.

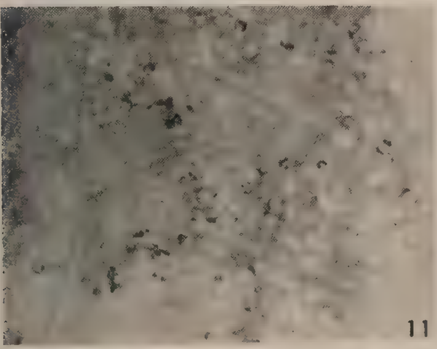
14 Fairly well preserved single-celled egg of Tethys (× 1000) showing Golgi bodies of the early vesicular type unassociated with yolk. Kolatchev technique, 5 μ.

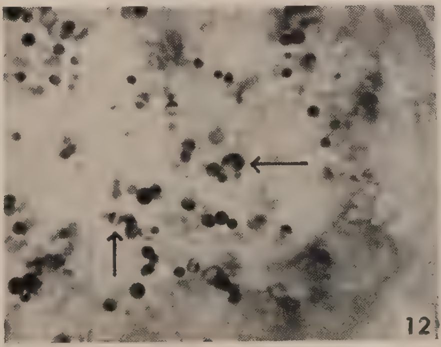

7

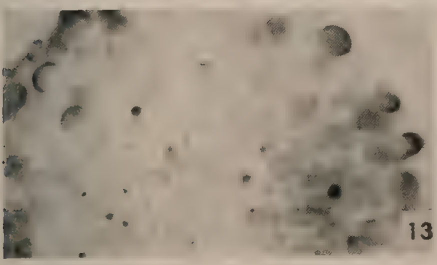

8


9


10


11


12


13

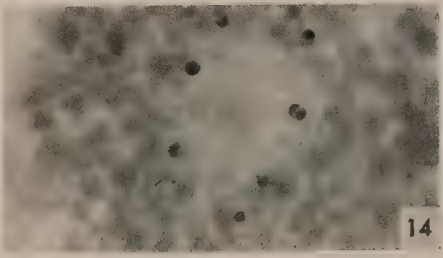

14

PLATE 4

EXPLANATION OF FIGURES

All figures on this plate are of material vitally stained with methylene blue.

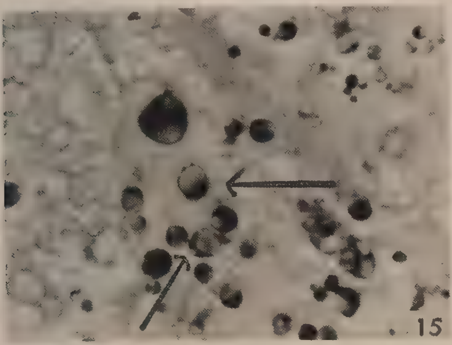
15 Golgi bodies of the active vesicular type (× 1000) in the early trochophore of Navanax. Photo taken at 3: 26 P.M. At diagonal arrow are Golgi bodies of the initial active phase (compare with fig. 1 g). An idea of the extent of the enlargement that has taken place will be gained by comparing with figures 7 and 12.

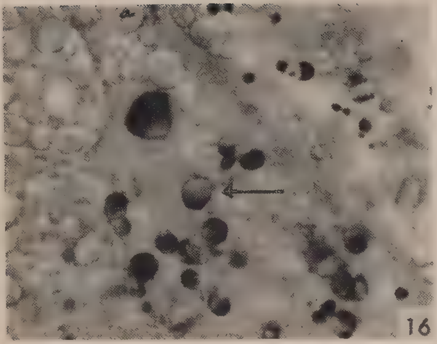
16 Same field as figure 15, taken at 3: 45 P.M. showing extent to which Golgi elements have changed in position and appearance. Note that the albuminous yolk sphere at (a), and most of the other yolk spheres have remained stationary, while many of the Golgi bodies, like the one at arrow have undergone rotation and other changes in appearance. An attempt was made to keep material at the same focus as figures 15 and 17.

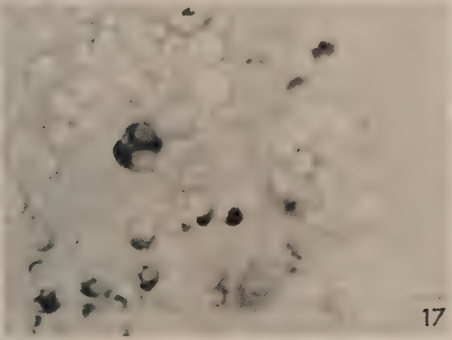
17 Same field as figures 15 and 16 at 4: 30 P.M. Desiccation of material is well under way producing Golgi artifacts of various sorts, but the yolk spheres appear unaltered.

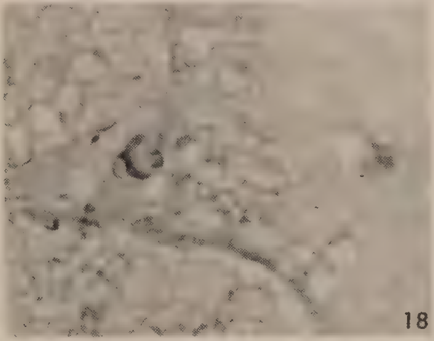
18 Same field as figures 15, 16 and 17 at 6: 15 P.M. Material completely desiccated on the slide. There has been considerable movement of yolk and other inclusions during desiccation and except for the large Golgi body, the respective inclusions cannot be identified with those of figure 17. Note the artificial appearance of the Golgi bodies as compared with those of figures 15, 16 and 17, but the unaltered condition of the yolk spheres.

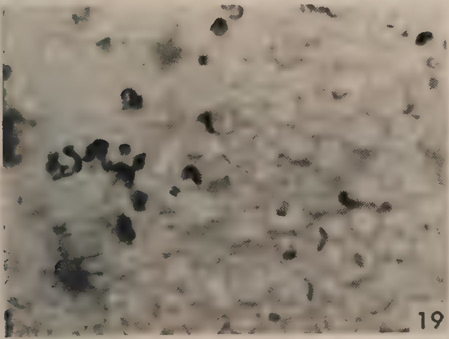
19, 20, 21 and 22 Other Golgi artifacts, loops, rings, rods and ''batonettes'', found elsewhere in similar material at a somewhat earlier stage in development. Note the poorly preserved ''chromophobe'' associated with most of the dictyosomes. At arrow in figure 22 is the only unaltered Golgi body in the four figures (× 1000).

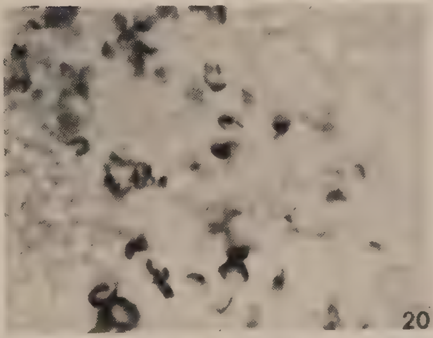

15

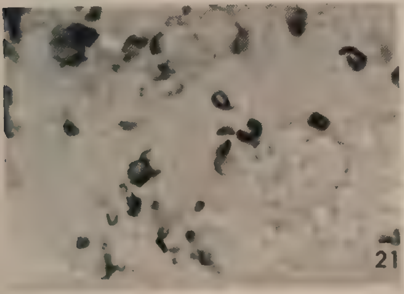

16


17


18


19


20


21

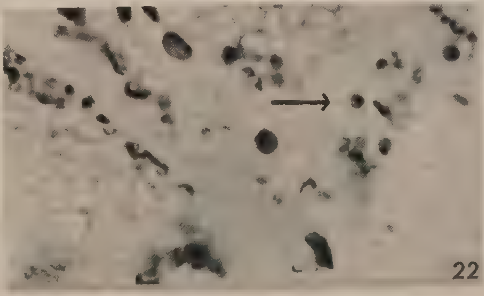

22

PLATE 5

EXPLANATION OF FIGURES

23 Parallel dictyosomes (× 1000) resembling those in figures 19, 22 and 24 seen in the fixed egg of Sabellaria. Compare with figure 24. Mann-Kopsch technique, 5 μ.

24 Parallel dictyosomes (× 1000) similar to those in figure 23 seen in the desiccated egg of Hermissenda. Vitally stained, before desiccation, with methylene blue.

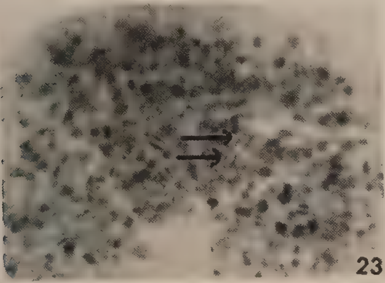
25 and 26 Fat synthesis and discharge (at arrows) by active Golgi bodies of the synthetic phase seen in the prototroch of the Navanax trochophore (× 1000). Vital methylene blue.

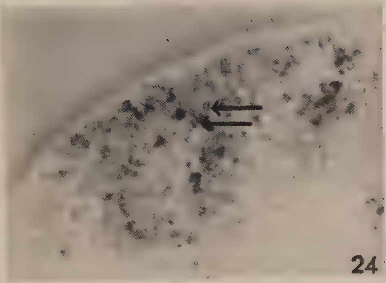
27 Living pycnotic Golgi bodies of the spiral of the early Navanax veliger (× 1000). Some droplet forms are visible. Vital methylene blue.

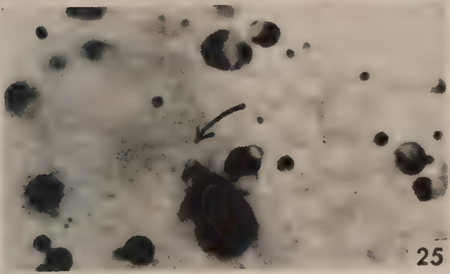
28 The Navanax veliger spiral (× 1000) a few hours later showing the resolution of the pycnotic Golgi bodies into droplets each of which is similar to the droplets of cleavage stages. Vital methylene blue.

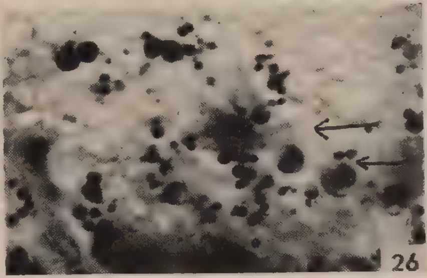
29 Discharge of albuminous yolk by pycnosis of Golgi bodies (× 1000). The arrows pointing downward indicate yolk discharge. The arrows pointing upward indicate the resolution into droplets following yolk discharge. The diagonal arrow points to a Golgi body where release of the yolk sphere is coincident with the division into droplets. One Golgi body adjacent to end of arrow has already attained the initial vesicular phase. Compare it with those of figure 7. Vital methylene blue.

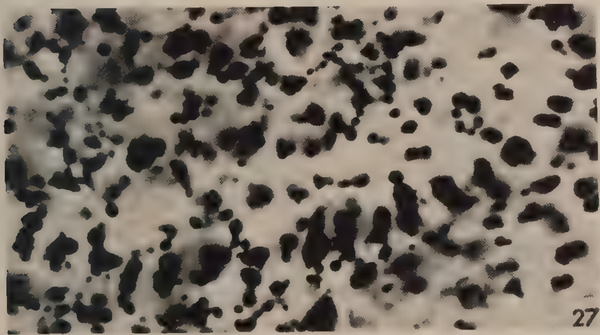
30 Navanax veligers 6 days old (× 100) stained supravitally with methylene blue in the cleavage stages. The large black spots are the heavily pigmented anal glands (?), while the small spots are the vitally stained Golgi bodies. A veliger spiral is seen at arrow.

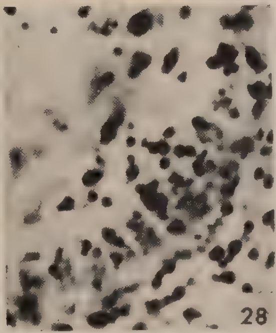

23

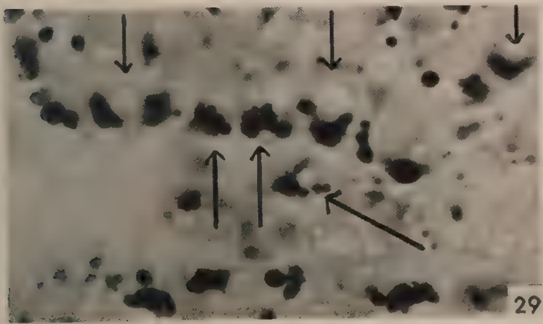

24

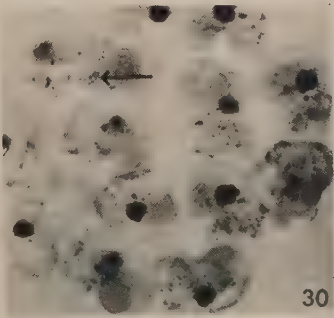

25


26


27


28


29


30

Contribution No. 212

Reprinted from TRANSACTIONS OF THE AMERICAN MICROSCOPICAL SOCIETY
Vol. LXII, No. 3, July, 1943

GYMNODINIUM CATENATUM, A NEW DINOFLAGELLATE FROM THE GULF OF CALIFORNIA*

HERBERT W. GRAHAM

Mills College, Oakland, California, and Scripps Institution of Oceanography, University of California, La Jolla, California

During the oceanographic investigations of the Scripps Institution in the Gulf of California in the spring of 1939 a rich plankton composed of a catenulate dinoflagellate was encountered. This was first believed to be *Gonyaulax catenella* Whedon and Kofoid and was so reported by Allen (1942). Later the identification was questioned. In their report of the Gulf Survey Gilbert and Allen (1942) refer to the organism as *Gymnodinium sp.* Careful examination of material kindly supplied me by Professor W. E. Allen reveals that the organism is a new specis of *Gymnodinium.*

The description below is based on material preserved in formaldehyde. Although few naked dinoflagellates withstand preservation this species is unusual in retaining fairly definite body shape. Some distortion has taken place as is evidenced by considerable variation in body contours. A description of the preserved material is justified, however, since the body shape and chain formation are so characteristic that the species can be easily recognized in preserved samples once an acquaintance with it is made.

Gymnodinium catenatum n. sp.

Dimensions. Length, 30 (22–33) μ; transdiameter, 36 (30–46) μ.

Description. Body circular to squarish in ventral view, truncate posteriorly with deep notch at sulcus, rounded anteriorly in anterior members of chains. The epicone of all other members of chains pointed where there is attachment to the sulcus of the cell ahead. In anterior view the body is subcircular.

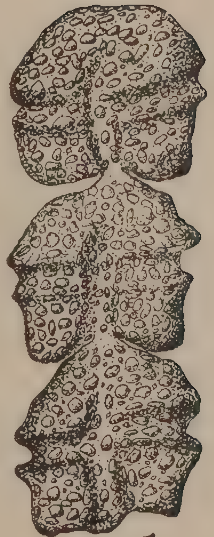
The species seems to occur in two forms. In one the length is approximately equal to the transdiameter; in the other there is considerable dorsoventral flattening so that the length is only about 0.64 times the transdiameter.

Girdle median, without displacement, wide, about 0.16 transdiameter, deeply impressed. Sulcus extends from near apex to antapex.

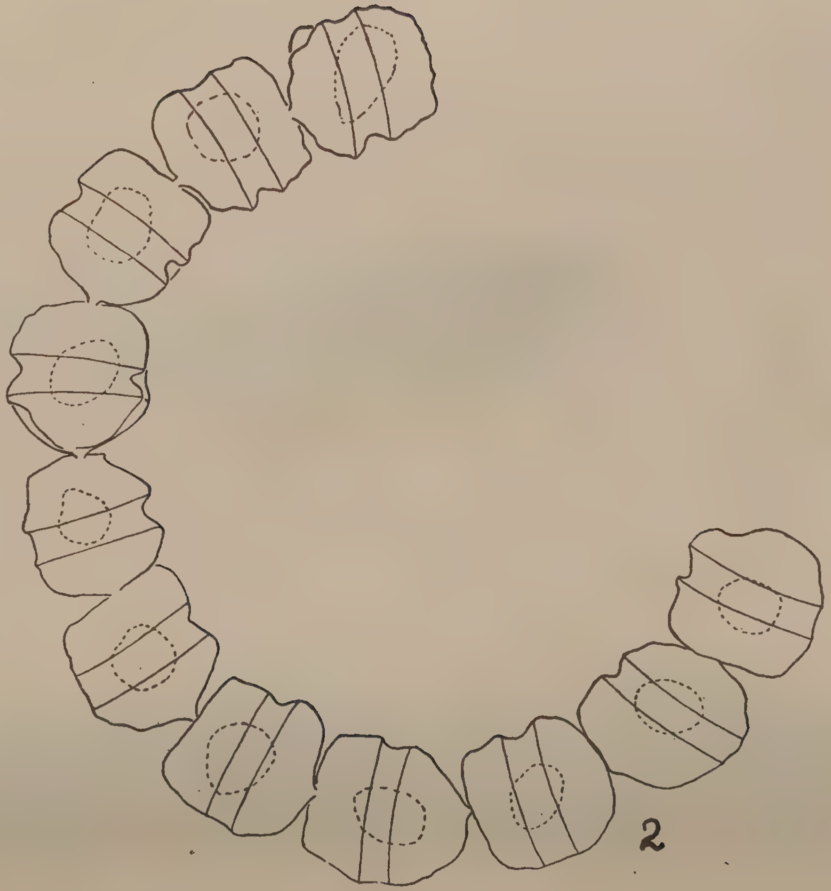
Cytoplasm greenish-yellow, packed with irregularly rounded refractory bodies and food vacuoles. Ectoplasm forms a thin pellicle in some stages. No striae or other surface markings observed. The protoplast has an unusual capacity to maintain its shape in formaldehyde preservative. This ability could not be ascribed to the presence of a firm pellicle since in many specimens it was impossible to demonstrate any semblance of pellicular structure.

---

* Contribution from the Scripps Institution of Oceanography, New Series No. 212.



1



2

Fig. 1. A three-celled chain of *Gymnodinium catenatum* n. sp. showing body contours and cytoplasmic structure. From Sample 4856, Station GC44. ×700.

Fig. 2. An eleven-celled chain of *Gymnodinium catenatum* n. sp. showing nuclei and characteristic curvature of chain. From Sample 4856, Station GC44. ×700.

The nucleus is centrally located, moniliform, large, thick, and slightly lunate.

The species occurs characteristically in chains of many individuals; as many as 29 units have been counted in a single chain. Many of the single individuals in preserved samples may represent broken chains. The cytoplasm is continuous throughout the chain. The apex of one individual is drawn out into a narrow extension which joins the posterior part of the sulcus of the next anterior member.

Occurrence. The species occurred at all stations north of 29° north latitude (Stations 41–52) in the Gulf of California. The greatest abundance was found at Station 51 where almost a million cells per liter occurred at the surface. At this station the species was responsible for the occurrence of "red water." Most large counts were found above 30 meters depth. In the region where the species occurred the surface temperature varied from 14° to 17°C.; the salinity from 35.07 to 35.50 o/oo. Collections were made in March, 1939.

The presence of green food vacuoles in the cells and the great abundance of the organism indicate that *Gymnodinium catenatum* was an important grazing organism in the Gulf in the spring of 1939. Whether it is always an important member of the plankton community in the upper Gulf cannot be stated at this time.

Comparisons. This species is a member of the subgenus *Gymnodinium* Kofoid and Swezy. Its closest relative is *G. uberrimum* (Allman) Kofoid and Swezy which has a similar body shape and shows a tendency toward chain formation. *G. catenatum* differs from this species in its smaller size, slightly lunate large nucleus, and lack of girdle displacement and radially arranged chromatophores. Its nearest marine relative is *G. dissimile* from which it is easily distinguished by the different shape of the epicone, smaller size, and strong tendency toward chain formation.

REFERENCES

Allen, W. E. 1942. Twenty years' statistical studies of marine plankton dinoflagellates of southern California. Amer. Midland Nat. 26: 603–635.

Gilbert, J. Y., and W. E. Allen. 1942. The phytoplankton of the Gulf of California obtained by the *E. W. Scripps* in 1939 and 1940. Jour. Mar. Res. (in press).

Contribution No. 213

"RED WATER" IN LA JOLLA BAY IN 1942

W. E. ALLEN

Reprinted from Transactions of the American Microscopical Society
Vol. LXII, No. 3, July, 1943

"RED WATER" IN LA JOLLA BAY IN 1942*

W. E. Allen

Scripps Institution of Oceanography of the University of California

Presumably, every kind of plant and animal inhabiting the sea has its days of extraordinary prosperity no less than do those organisms inhabiting the land. Of course, we know very well from our own observation that most dwellers on land rarely, or never, become conspicuous at any of their greatest extremes of success in growth and multiplication, sometimes because they are too small but more often because a speed of approach to prominence natural to them is too low to permit conspicuous expansion before conditions become unfavorable for them. Because it is usually difficult for us to observe things in the sea, it is evident that still fewer kinds of plants or animals have opportunities to become conspicuous there.

While it is true that exceptional numbers of fishes or other marine animals may be seen *in* the sea water from a position more or less high above the surface under favorable circumstances, it is fair to say that ordinarily our attention is attracted to large abundances in only two ways, i.e., disturbance of the surface or the appearance of unfamiliar color of the water. So far as I have noticed, in more than 20 years of acquaintance with La Jolla Bay, porpoises and sardines have furnished the best examples of agitation of the surface, the former by their large splashes and the latter by their ripply or frothy agitation of the surface. In that same period all of the conspicuous discolorations have been caused by microscopic plants called dinoflagellates.

In Southern California, it is reasonable to suppose that all discolorations of the sea by microscopic plants can be included under the popular designation "red water" although some of the shades and tints are not really red. Indeed, they have been observed in ranges from light greenish or lemon yellow up to a fairly dark brown. However, it is difficult to make a clear distinction, most particularly because many of the shades of color can be seen within a few yards of each other when it is known that only one kind of plant is giving the color. Where the tiny plants are highly concentrated, perhaps 300 or 400 to each drop of water, the color is much deeper than it is nearby where the concentration is no greater than 100 or less to each drop.

On September 11, 1942, I noticed that a fairly large body (cloud) of "red water" was coming inshore and appeared likely to pass under the outer end of the pier of the Scripps Institution of Oceanography. It seemed to be a good opportunity to get a sample easily but I waited nearly an hour without much success. The deeply colored water remained just out of my reach, although it moved inshore slightly on both sides of the pier, about 10 yards away on the north side and 10 feet away on the south. The only sample obtained was from the margin of the cloud. Seeming faintly tinged with brownish red as I looked down on it, it showed no color at all when I had hauled up a pail full for close observation. In the laboratory I found that the numbers were fairly large, nearly 200,000 cells per liter.

---

* Contributions from the Scripps Institution of Oceanography, New Series, No. 213.

This particular microscopic plant was *Gonyaulax polyedra* Stein, which carries a doubtful, if not thoroughly bad, reputation because it has been accused of killing off entire populations of animals dwelling inshore in Southern California, most notably near San Pedro in 1917. This reputation is not improved by kinship of the organism with *G. catanella* Whedon and Kofoid which is held responsible for many human deaths through "mussel poisoning."

G. polyedra is very erratic in occurrence. Not only does it rarely reach an abundance sufficient to cause "red water" but it has been entirely missing from records from daily catches at the Scripps Institution pier for years at a time. Since 1917 it has reached conspicuous abundance in La Jolla Bay in only three years, 1932, 1938, and 1942. To people who look upon the sea only as a mass of water and as a place in which nothing ever happens this surely will indicate highly interesting possibilities. Perhaps, after all, there is a constant struggle there between living things and each other and between them and "Nature." Some far reaching influence or combination of influences must have been responsible for the sudden appearance of such enormous populations in three years out of 25.

In the general region of San Diego another dinoflagellate has caused more or less appearance of "red water" far more frequently than *G. polyedra* through a period of nearly thirty years. Indeed, there have been few years since 1916 in which it has not made at least a slight showing at some time in the season from April to September. It is called *Prorocentrum micans* Ehrenberg. Most often it has done no more than cause a yellowish froth to appear in the crests of wavelets or in irregular streaks on the surface of relatively quiet water off shore a short distance. But in a few years its populations have developed to density causing a deeply red color like that of *G. polyedra*. A single lively cell of *P. micans* has a beautiful color of soft golden yellow. The relatively ugly red of the dense populations is due entirely to the combined effect of the masses of enormous numbers, just as thick paint is different in appearance from paint thinly spread. Fairly large populations of *P. micans* were developed in La Jolla Bay in May, 1942 but they were not large enough to cause conspicuous "red water."

Here are some tantalizing biological problems. Not only is it difficult to think of any good reason why *G. polyedra* should reach "red water" abundance in La Jolla Bay in only three years out of nearly 30, especially when it has been missing in several years and nearly missing in several more, but it is still more difficult to see how it contrived to do so in a region much more regularly populated by *P. micans*. However, interesting as are these and a number of other biological conditions, they are far from constituting the only items or questions of importance. Briefly, it may be noted that numerous physical and chemical conditions have had influences more or less directly effective, most of them capable of identification and evaluation if the history of Southern California seas could have been followed in full detail throughout the period. Naturally, movements of water masses (currents) and air masses come first to mind, but it should be evident that distribution of light and temperatures in both water and air are distinctly influential. Then there are conditions of local and general turbulence, density, viscosity, oxygen content, and numerous chemical solutions and suspensions. Surely we must believe that in certain years and seasons the combinations of these and many other influences were not right at any time that the particular tiny plants were ready for enormous increase in numbers,but that in certain other years and seasons the whole series of favorable combinations did happen to occur together (or nearly enough together) to result in the amazing abundances which we call "red water."

Contribution No. 214

Reprinted from SCIENCE, September 3, 1943, Vol. 98, No. 2540, pages 208-209.

IMAGINARY SUBMARINE CANYONS[1]

By Dr. FRANCIS P. SHEPARD
DIVISION OF WAR RESEARCH, UNIVERSITY OF CALIFORNIA, SAN DIEGO, CALIF.

THE VEATCH-SMITH SUBMARINE CONTOURS

FOR some years the discovery of the great submarine canyons along the oceanic slopes off the east coast filled many of the outstanding geological theorists with misgivings. They were less disturbed by the deeply submerged canyons with the characteristics of river valleys off California, since this coast was well known to be unstable, but river canyons off the supposedly stable east coast were unthinkable; and to make matters worse, charts were showing that submarine canyons were practically universal. Assuredly the continents of the world could not all have been moving up and down many thousands of feet during the late part of geological time. Into the breech at the critical moment stepped a series of maps of outstanding workmanship which appear to have taken at least the eastern part of the country by storm. These maps were the result of painstaking contouring by A. C. Veatch and by his collaborator, Paul Smith.[2] The contours, as both Veatch and Smith have frankly admitted, are based on the hypothesis that the submarine slopes have a stream-cut type of topography. Accordingly, the contours show a great mass of gullies and valleys even out at the greatest depths where there are few soundings. These small valleys shown on the maps have been seized on eagerly without any apparent attempt to determine their basis and are now established as the pièce de resistance of three new hypotheses advocating submarine origin of the canyons. Two of these have appeared in books[3] and a third as a short article.[4] The maps have also been reproduced in many textbooks and have been the source from which a model reposing in the American Museum of Natural History was drawn. Even *Life* magazine recently reproduced a picture of this model with mention in garbled form of one of the new hypotheses.

Since the tendency of late has been to regard these gullies as the typical submarine valleys and accordingly to disregard the great rock-walled canyons which have been revealed by years of scientific exploration of the sea floor, it might be well to give the scientific public some insight on the basis of these supposed gullies and their relation to the problem of the history of the ocean floors. Regarding these contours one must admit in all fairness that they represent long painstaking effort. Certainly it would have been difficult to fit stream-dissected slope topography more perfectly into the soundings than has been done. On the other hand, the contouring could have been executed with the same care in attempting to show what actually existed out on these submarine slopes off the east coast. That this was not done is indicated by the fact that the same soundings were contoured by a series of six topographers and geologists of the U. S. Geological Survey and by the present writer and his co-worker, K. O. Emery, and in no case did these closely spaced small valleys appear in the contoured maps which resulted. Instead of these myriads of gullies a series of relatively large canyons (shown also by Veatch and Smith) are revealed on the slopes. The canyons are of the type which recent theories have so meticulously avoided. In a forthcoming paper, Emery and the writer will present in some detail the abundant evidence which is thought to reject the gully hypothesis of contour interpretation of these soundings off the east coast. It may be sufficient to state here that any one who has a conviction of the existence of these gullies need only return to the original data and attempt to contour some portion of the deep outer slopes bearing in mind that unavoidable errors of observation increase with depth.

A SUBMARINE ORIGIN OF THE CANYONS

The recent hypotheses which attempt to explain submarine canyons as formed without emergence include in order of their appearance: R. A. Daly's muddy density currents, D. W. Johnson's artesian springs, and W. H. Bucher's tsunamic sea waves (also called tidal waves or seismic sea waves). In defending these hypotheses the advantage of the gully concept provided by the Veatch-Smith maps is very evident, since none of these processes could account for huge canyons. All these authors have referred to canyons like those off the California coast as a different phenomenon from the submarine valleys which they are trying to explain. They all imply that submarine valleys in general are of the nature of the Veatch-Smith gullies.

---

[1] Contributions from the Scripps Institution of Oceanography, New Series, No. 214.

[2] Special Paper 7, Geological Society of America, 1939.

[3] D. W. Johnson, "The Origin of Submarine Canyons," Columbia University Press, 1939; R. A. Daly, "The Floor of the Ocean," Univ. of North Carolina Press, 1942.

[4] W. H. Bucher, *Bull. Geol. Soc. Amer.*, Vol. 51, pp. 489–512.

Daly's idea that muddy currents moved down the ocean slopes during glacial stages might conceivably be used to explain small gullies cut into a muddy slope, but even for this explanation Daly had to draw on the now discredited idea that the outer continental shelves are or were great mud banks. Bucher's tsunamis (tidal waves) must set up occasional strong currents on the ocean floor, but it would be amazing if these rare manifestations could have cut more than small valleys into the ocean slopes. Furthermore, the distribution of submarine canyons shows no relationship to the areas where these waves occur. Also the waves are of a nature which should produce erosion on projections rather than in valley-like indentations. Johnson's artesian springs require high land along the coast with water-bearing layers dipping seaward and extending under the shelf and outcropping on the outer slopes. Even under these ideal and rare conditions, excavations on the slope by the artesian springs could produce only small valleys, which should have many basin depressions along their length. Even the fanciful Veatch-Smith contours show nothing of this sort. For further objections to these hypotheses the reader may refer either to the Johnson or Daly book which are most successful in discounting the other's hypothesis or to the writer's discussion of the subject in which an attempt was made to give facts favorable and unfavorable to each hypothesis.[5]

LEAVING HAMLET OUT OF THE PLAY

If all this attempt to explain submarine valleys is dealing with a purely fictitious concept, the situation would appear to approach the ridiculous. Are the typical submarine valleys of the world of the order of these hypothetical east coast gullies or are they comparable to the well-known California rock-walled canyons? All the available evidence suggests that the latter is the case. The Coast and Geodetic Survey charts provide abundant evidence of the existence of canyons thousands of feet deep and extending many miles into the outer edge of the continental shelf. H. C. Stetson's well-known investigations have shown that many of the sea canyons have rocky walls. Most of the information regarding other submarine valleys comes from the charts of the world. All available charts have been studied by the writer and the soundings indicate clearly that the great majority of the known submarine valleys are large canyons, that is, canyons comparable in dimensions with those which cut great mountain walls. Furthermore, the bottom notations reveal that rock is present on the canyon sides in numerous places. These statements may be easily verified by examination of the charts from the various coasts of the world.

Is it reasonable to present a hypothesis which disregards such great submarine canyons as the huge Hudson Canyon which can be traced for miles off New York, the canyon off the Congo, which is a hundred and twenty miles long and extends 18 miles into the coast in the Congo estuary, the huge Fosse de Cap Breton off southwestern France, the equally large Canal de Nazare off the Portuguese coast which appears to be cut in granite, the canyon off the Indus which crosses a 60-mile shelf off the Indus River or the rock-walled canyons off Ceylon, to mention a few all off relatively stable coasts? If we consider all these great canyons as well as all the canyons off unstable coasts as exceptional, just what will we have left? We are not only excluding Hamlet from the stage, but all the other actors.

RIVER EROSION

Despite the enthusiastic abandonment of the river erosion hypothesis by these recent writers the facts continue to favor the idea. All the detailed surveys of the submarine canyons have revealed distinctly river valley characteristics.[6] Characteristically these canyons the world over are V-shaped, steep rock-walled, winding valleys with branching tributaries. A very large per cent. are located off river mouths and some of them are obviously submarine continuations of land canyons. The explored canyons off California contain numerous rounded pebbles even at depths as great as one mile below sea level. Many other observations indicate the submergence of the land margins. The case may not be rigidly established, but it is substantial and should not be thrown aside just because it presents a difficulty in the path of certain preconceived hypotheses. It is to be hoped that if there are future suggestions of causes of submarine canyons, they will be evolved after a careful study of the facts or preferably after field investigation of the canyons.

---

[5] Special Paper No. 31, Geological Society of America, 1941.

[6] Charts of many are available in Special Paper 31, Geological Society of America.

LIST OF CONTRIBUTIONS

from the

SCRIPPS INSTITUTION OF OCEANOGRAPHY

NEW SERIES

Number

1. SVERDRUP, H. U. On the evaporation from the oceans. *Jour. Marine Research*, v. 1, p. 3-14, 1937.
2. ZOBELL, C. E. Studies on the bacterial flora of marine bottom sediments. *Jour. Sed. Petrol.*, v. 8, p. 10-18, 1938.
3. ALLEN, W. E. Non-visible specimens. *Turtox News*, v. 16, p. 1-3, 1938.
4. SVERDRUP, H. U. Notes on erosion by drifting snow and transport of solid material by sea ice. *Amer. Jour.. Sci.*, v. 35, p. 370-73, 1938.
5. ZOBELL, C. E., and S. C. RITTENBERG. The occurrence and characteristics of chitinoclastic bacteria in the sea. *Jour. Bacter.*, v. 35, p. 275-87, 1938.
6. NATLAND, M. L. New species of foraminifera from the west coast of North America and from later Tertiary of the Los Angeles Basin. *SCRIPPS INSTITUTION BULLETIN*, tech. ser., v. 4, p. 137-64, 1938.
7. LAFOND, E. C. Relationship between mean sea level and sand movement. *Science*, v. 88, p. 112-13, 1938.
8. MYERS, E. H. The present state of our knowledge of the life history of the foraminifera. *Nat. Acad. Sci., Proc.*, v. 24, p. 10-17. 1938.
9. SUMNER, F. B., and P. DOUDOROFF. Some experiments upon temperature acclimatization and respiratory metabolism in fishes. *Biol. Bull.*, v. 74, p. 403-29, 1938.
10. YOUNG, R. T. The effect of balanced versus unbalanced solutions of pH and distilled water on fish. *Ecology*, v. 19, p. 126-40, 1938.
11. MCEWEN, G. F. Modern dynamical oceanography: an achievement of application to ocean observations of principles of mechanics and heat. *Philos. Soc., Proc.*, v. 79, p. 145-66, 1938.
12. ALLEN, W. E. Quantity collecting of planktonic diatoms. *Science*, v. 87, p. 171-72, 1938.
13. SHEPARD, F. P., and G. E. MACDONALD. Sediments of Santa Monica Bay, California. *A. A. P. G., Bull.*, v. 22, p. 201-16, 1938.
14. CUPP, E. E., and W. E. ALLEN. Plankton diatoms of the Gulf of California. *Allan Hancock Pacific Exped.*, v. 3. p. 61-99, 1938.
15. SVERDRUP, H. U. On the process of upwelling. *Jour. Marine Research*, v. 1, p. 155-64, 1938.
16. ZOBELL, C. E., and H. D. MICHENER. A paradox in the adaptation of marine bacteria to hypotonic solutions. *Science*, v. 87, p. 328-29, 1938.
17. FOX, D. L, and C. R. MOE. An Astacene-like carotenoid from a Pacific Coast anemone, *Epiactis prolifera*. *Nat. Acad. Sci., Proc.*, v. 24, p. 230-35, 1938.

Number

18. Gordon, R. D. Note on estimating bacterial populations by the dilution method. *Nat. Acad. Sci., Proc.*, v. 24, p. 212-15, 1938.

19. Young, R. T. The life history of a trematode (*Levinseniella cruzi?*) from the shore birds (*Limosa fedoa* and *Catoptrophorus semipalmatus inornatus*). *Biol. Bull.*, v. 74, p. 319-39, 1938.

20. Johnson, M. W. Concerning the copepod *Eucalanus elongatus* Dana and its varieties in the northeast Pacific. *SCRIPPS INSTITUTION BULLETIN, tech. ser.*, v. 4, p. 165-80, 1938.

21. Allen, W. E. "Red water" along the west coast of the United States in 1938. *Science*, v. 88, p. 55-56, 1938.

22. Sverdrup, H. U. On the explanation of the oxygen minima and maxima in the oceans. *Jour. du Conseil*, v. 13, p. 163-172, 1938.

23. Sverdrup, H. U. Research within physical oceanography and submarine geology at the Scripps Institution of Oceanography during April 1937 to April 1938. *Amer. Geophys. Union, Trans.*, p. 238-42, 1938.

24. Fleming, R. H. Tides and currents in the Gulf of Panama. *Jour. Marine Research*, v. 1, p. 192-206, 1938.

25. McEwen, G. F. Some energy relations between the sea surface and the atmosphere. *Jour. Marine Research*, v. 1, p. 217-38, 1938.

26. Monk, C. R. An aqueous medium for mounting small objects. *Science*, v. 88, p. 174-76, 1938.

27. Evans, R. D., A. F. Kip, E. G. Moberg. The radium and radon content of Pacific Ocean water, life and sediments. *Amer. Jour. Sci.*, v. 36, p. 241-59, 1938.

28. Ritter, W. E. Dr. Fred Baker. *Science*, v. 88, p. 48-49, 1938.

29. Coe, W. R. Influence of association on the sexual phases of gastropods having protandric consecutive sexuity. *Biol. Bull.*, v. 75, p. 274-85, 1938.

30. Marks, G. W. The copper content and copper tolerance of some species of mollusks of the southern California coast. *Biol. Bull.*, v. 75, p. 224-37, 1938.

31. Sumner, F. B., and P. Doudoroff. Some effects of light intensity and shade of background upon the melanin content of Gambusia. *Nat. Acad. Sci.*, v. 24, p. 459-63, 1938.

32. Sumner, F. B., and P. Doudoroff. The effects of light and dark backgrounds upon the incidence of a seemingly infectious disease in fishes. *Nat. Acad. Sci., Proc.*, v. 24, p. 463-66, 1938.

33. Coe, W. R. Sexual phases in the gastropod *Crucibulum spinosum*. *Jour. Morphology*, v. 63, p. 345-61, 1938.

34. Allen, W. E. The Templeton Crocker Expedition to the Gulf of California in 1935—The phytoplankton. *Amer. Microsc. Soc., Trans.*, v. 57, p. 328-35, 1938.

Number

35. ZoBell, C. E. The sequence of events in the fouling of submerged surfaces. *Official Digest, Fed'n Paint and Varnish Prod. Clubs*, no. 178, p. 379-85, 1938.

36. Coe, W. R. A new genus and species of Hoplonemertea having differential polar sexuality. *Zoologischer Anzeiger*, v. 124, p. 220-24, 1938.

37. Gordon, R. D., and C. E. ZoBell. Note on the successive dilution method for estimating bacterial population. *Zentralbl. f. Bakteriol.*, II Abt., Bd. 99, p. 318-20, 1938.

38. Fox, D. L. An illustrated note on the mating and egg-brooding habits of the two-spotted octopus. *San Diego Soc. Nat. Hist., Trans.*, v. 9, p. 31-34, 1938.

39. Shepard, F. P., and C. E. LaFond. Undertow—A discussion. *Science*, v. 89, p. 78-76 1938.

40. ZoBell, C. E., and C. B. Feltham. Bacteria as food for certain marine invertebrates. *Jour. Marine Research*, v. 1, p. 312-27, 1938.

41. ZoBell, C. E., and J. H. Long. Studies on the isolation of bacteria-free cultures of marine phytoplankton. *Jour. Marine Research*, v. 1, p. 328-34, 1938.

42. Doudoroff, Peter. Reactions of marine fishes to temperature gradients. *Biol. Bull.*, v. 75, p. 494-509, 1938.

42-A. Fox, D. L., and M. C. Sargent. Variations in chlorophyll and carotenoid pigments of the brine flagellate, *Dunaliella salina*, induced by environmental concentrations of sodium chloride. *Chem. and Industry*, v. 57, p. 1111, 1938.

43. Allen, W. E. Surface distribution of marine plankton diatoms in the Panama region in 1933. *SCRIPPS INSTITUTION BULLETIN, tech, ser.*, v. 4, p. 181-96, 1939.

44. Emery, K. O. Rapid method of mechanical analysis of sands. *Jour. Sed. Petrol.*, v. 8, p. 105-111, 1938.

45. Sverdrup, H. U. Second note on the logarithmic law of wind structure near the ground. *Royal Meteor. Soc., Quart. Jour.*, v. 65, p. 57-60, 1939.

46. ZoBell, C. E. The biological approach to the preparation of anti-fouling paints. *Sci. Section, Nat'l Paint, Varnish and Lacquer Assoc., Proc.*, Circular 588, p. 149-63, 1939.

47. Sverdrup, H. U. On the influence of stability and instability on the wind profile and the eddy conductivity near the ground. *Fifth Intern. Congr. Applied Mech., Trans.*, p. 369-72, 1939.

48. Sverdrup, H. U. Oceanic circulation. *Idem*, p. 279-293, 1939.

49. Sumner, F. B. Quantitative effects of visual stimuli upon pigmentation. *Amer. Naturalist*, v. 73, p. 219-34, 1939.

50. Sumner, F. B. Human psychology and some things that fishes do. *Scientific Monthly*, v. 49, p. 245-55, 1939.

51. Allen, W. E. Growth of a marine observatory. *Intern. Rev. d. ges. Hydrob. u. Hydrog.*, Bd. 39, p. 464-71, 1939.

Number

51-A. Fox, D. L., and C. F. A. Pantin. The biochemical basis of the colors of the plumose Anemone, *Metridium senile*. *Anat. Record*, v. 75, Suppl., p. 135-36, 1939.

52. Sverdrup, H. U. Physics and geophysics. *Univ. California Faculty Research Lecture*, 23 pp., 1939.

53. Shepard, F. P., R. Revelle, R. S. Dietz. Ocean-bottom currents off the California coast. *Science*, v. 89, p. 488-89, 1939.

54. Gordon, R. D. Some preliminary postulates on stimulus-perception, and consequent probability distribution for behavior. *Psychometrika*, v. 4, p. 89-98, 1939.

55. Coe, W. R. Sexual phases in terrestrial Nemerteans. *Biol. Bull.*, v. 76, p. 416-27, 1939.

56. Allen, W. E. Microcopepoda in marine phytoplankton catches. *Science*, v. 89, p. 532-33, 1939.

57. LaFond, E. C. Variations of sea level on the Pacific Coast of the United States. *Jour. Marine Research*, v. 2, p. 17-29, 1939.

58. Allen, W. E. Cut-throat competition in the sea. *Scientific Monthly*, p. 111-19, 1939.

59. Fleming, R. H. Tables for Sigma-T. *Jour. Marine Research*, v. 2, p. 9-11, 1939.

60. Gordon, R. D. (with foreword by C. E. ZoBell). Estimating bacterial populations by the dilution method. *Biometrika*, p. 167-80, 1939.

61. Young, R. T., Jr., and R. D. Gordon. Report on the penetration of light in the Pacific Ocean off the coast of southern California. *SCRIPPS INSTITUTION BULLETIN, tech. ser.*, v. 4, p. 197-218, 1939.

62. Scheer, B. T. The development of the concept of tissue respiration. *Annals of Science*, v. 4, p. 295-305, 1939.

63. Sverdrup, H. U. Research within physical oceanography and submarine geology at the Scripps Institution of Oceanography during April 1938 to April 1939. *Amer. Geophys. Union, Trans.*, p. 422-27, 1939.

64. Johnson, M. W. *Pseudodiaptomus* (*Pseudodiaptallous*) *euryhalinus*. A new subgenus and species of Copepoda, with preliminary notes on its ecology. *Amer. Micros. Soc., Trans.*, v. 58, p. 349-55, 1939.

65. Fleming, R. H. The control of diatom populations by grazing. *Cons. Perm. Intern., Jour. du Conseil*, v. 14, p. 210-27, 1939.

66. a. Vaughan, T. W. Presentation of the Agassiz Medal to Harald Ulrik Sverdrup. *Science*, v. 90, p. 24-26, 1939. b. Sverdrup, H. U. Response of the Medallist, *Idem*, p. 26-27, 1939.

67. ZoBell, C. E. Primary film formation by bacteria and fouling. *Collecting Net*, v. 14, 2 pp., 1939.

68. Young, R. T., Jr. Measurements on the transparency of sea-water off the coast of southern California. *Jour. Marine Research*, v. 2, p. 117-125, 1939.

Number

69. Sverdrup, H. U., and W. E. Allen. Distribution of diatoms in relation to the character of water masses and currents off southern California. *Jour. Marine Research*, v. 2, p. 131-44, 1939.

70. Fleming, R. H., and Roger Revelle. Physical processes in the ocean. In: *Recent Marine Sediments, A symposium*, p. 48-141, A.A.P.G., Tulsa, Oklahoma, 1939.

71. Revelle, Roger, and F. P. Shepard. Sediments off the California coast. *Idem*, p. 245-82, 1939.

72. ZoBell, C. E. Occurrence and activity of bacteria in marine sediments. *Idem*, p. 416-27, 1939.

73. Scheer, B. T. "Homing instinct" in salmon. *Quart. Rev. Biol.*, v. 14, p. 408-30, 1939.

74. Allen, W. E. "Phosphorescence" in the sea. *Nautical Gazette*, 2 pp., Aug. 26, 1939.

75. Sverdrup, H. U. Lateral mixing in the deep water of the South Atlantic Ocean. *Jour. Marine Research*, v. 2, p. 195-207, 1939.

76. Jacobs, W. C. A discussion of physical factors governing the distribution of microorganisms in the atmosphere. *Idem*, v. 2, p. 218-24, 1939.

77. Rittenberg, Sydney. Investigations on the microbiology of marine air. *Idem*, v. 2, p. 208-17, 1939.

78. Jacobs, W. C. Sea-level departures on the California coast as related to the dynamics of the atmosphere over the North Pacific Ocean. *Idem*, v. 2, p. 181-94, 1939.

79. Johnson, M. W. The correlation of water movements and dispersal of pelagic larval stages of certain littoral animals, especially the sand crab, *Emerita*. *Idem*, v. 2, p. 236-45, 1939.

80. Coe, W. R. Notes on the morphology and sexuality of the terrestrial Nemertean *Geonemertes palaensis*. *Bishop Museum, Occas. Papers*, p. 205-11, 1940.

81. Sumner, F. B., and M. C. Sargent. Some observations on the physiology of warm spring fishes. *Ecology*, v. 21, p. 45-54, 1940.

82. Sumner, Francis B. Further experiments on the relations between optic stimuli and the increase or decrease of pigment in fishes. *Jour. Exper. Zool.*, v. 83, p. 327-43, 1940.

83. Shepard, Francis P., and Eugene C. LaFond. Sand movements along the Scripps Institution pier. *Amer. Jour. Science*, v. 238, p. 272-85, 1940.

84. Sverdrup, H. U. The currents of the Pacific Ocean and their bearing on the climates of the coasts. *Science*, v. 91, p. 273-82, 1940.

85. Coe, W. R. Revision of the Nemertean fauna of the Pacific coasts of North, Central, and northern South America. *Allan Hancock Pacific Exped.*, v. 2, p. 247-323, 1940. (*Not available for exchange.*)

86. Allen, W. E. Life in a drop of sea water. *Turtox News*, v. 18, no. 4, 3 pp., 1940.

Number

87. Emerson, Ralph, and Denis L. Fox. Gamma-carotene in the sexual phase of the aquatic fungus *Allomyces*. *Roy. Soc. London, Proc., Ser. B, No. 852*, v. 128, p. 275-293, 1940. (*Not available for exchange.*)

88. ZoBell, Claude E., and Janice Stadler. The effect of oxygen tension on the oxygen uptake of lake bacteria. *Jour. Bacteriology*, v. 39, p. 307-22, 1940.

89. ZoBell, Claude E., and Janice Stadler. The oxidation of lignin by lake bacteria. *Archiv. f. Hydrobiol.*, Bd. 37, p. 163-71, 1940. (*Not available for exchange*).

90. Sverdrup, H. U. On the annual and diurnal variation of the evaporation from the ocean. *Jour. Marine Research*, v. 3, p. 93-104, 1940.

91. Sverdrup, H. U. Hydrology, Section 2, Discussion. *B.A.N.Z. Antarctic Research Expedition 1921-31, Repts., Ser. A*, v. 3, *Oceanography*, part 2, *Hydrology*, Sect. 2, p. 88-126, 1940. (*Not available for exchange.*)

92. ZoBell, Claude E., and Jean Conn. Studies on the thermal sensitivity of marine bacteria. *Jour. Bacteriology*, v. 40, p. 223-38, 1940.

93. Lyman, John, and Richard H. Fleming. Composition of sea water. *Jour. Marine Research*, v. 3, p. 134-46, 1940.

94. Sverdrup, H. U. Research within physical oceanography and submarine geology at the Scripps Institution of Oceanography during April 1939 to April 1940. *Amer. Geophys. Un., Trans.*, 1940, p. 343-46, 1940.

95. Sverdrup, H. U. Trail blazing in the Pacific. (Research in oceanography). *California Monthly*, p. 10, 11, 28, Dec., 1940. Berkeley.

96. ZoBell, Claude E. Some factors which influence oxygen consumption by bacteria in lake water. *Biol. Bull.*, v. 78, p. 388-402, 1940.

97. Sumner, Francis B. The naturalist as a social phenomenon. *Amer. Naturalist*, v. 47, p. 398-408, 1940.

98. Sumner, Francis B. Quantitative changes in pigmentation, resulting from visual stimuli in fishes and amphibia. *Biol. Reviews*, v. 15, p. 351-75, 1940.

99. Allen, W. E. Discolored sea water. *U. S. Hydrographic Bull.*, no. 2262, 3 pp., 1940.

100. Allen, W. E. Indicator value of phytoplankton at the Scripps Institution. *Sixth Pac. Sci. Congr., California, 1939, Proc.*, v. 3, p. 529-531, 1940-41.

101. Allen, W. E. Methods of field and laboratory procedure in phytoplankton research at the Scripps Institution of Oceanography. *Sixth Pac. Sci. Congr., California, 1939, Proc.*, v. 3, p. 525-528, 1940-41.

102. Allen, W. E. Summary of results of twenty years of researches on marine phytoplankton. *Sixth Pac. Sci. Congr., California, 1939, Proc.*, v. 3, p. 577-583, 1940-41.

103. Fleming, Richard H. Character of the currents off southern California. *Sixth Pac. Sci. Congr., California, 1939, Proc.*, v. 3, p. 149-160, 1940-41.

Number

104. Fleming, Richard H. Composition of plankton and units for reporting populations and production. *Sixth Pac. Sci. Congr., California, 1939, Proc.*, v. 3, p. 535-540, 1940-41.

105. Fleming, Richard H. A contribution to the oceanography of the Central American region. *Sixth Pac. Sci. Congr., California, 1939, Proc.*, v. 3, p. 167-175, 1940-41.

106. Johnson, Martin W. The study of species formation in certain Eucalanus copepods in the North Pacific. *Sixth Pac. Sci. Congr., California, 1939, Proc.*, v. 3, p. 565-568, 1940-41.

107. La Fond, Eugene C. Sand movements near the beach in relation to tides and waves. *Sixth Pac. Sci. Congr., California, 1939, Proc.*, v. 2, p. 795-799, 1940.

108. Sargent, Marston C. A theoretical definition of production. *Sixth Pac. Sci. Congr., California, 1939, Proc.*, v. 3, p. 513-516, 1940-41.

109. Sverdrup, H. U. The Gulf of California: Preliminary discussion of the cruise of the *E. W. Scripps* in February and March, 1939. *Sixth Pac. Sci. Congr., California, 1939, Proc*, v. 3, p. 161-166, 1940-41.

110. Sverdrup, H. U. Activities of the Scripps Institution of Oceanography ⟦1933-1938⟧. *Sixth Pac. Sci. Congr., California, 1939, Proc.*, v. 3, p. 114-123, 1940-41.

111. Shepard, Francis P. Submarine geology and geophysics. *Nature*, v. 146, p. 431-432, 1940.

112. Scheer, Bradley T. Some features of the metabolism of the carotenoid pigments in the California sea mussel (*Mytilus californianus*). *Jour. Biol. Chem.*, v. 136, p. 275-299, 1940.

113. Sverdrup, H. U. The unity of the sciences of the sea. *Sigma Xi Quart.*, v. 28, p. 105-115, 1940.

114. Rittenberg, Sydney C. Bacteriological analysis of some long cores of marine sediments. *Jour. Marine Research*, v. 3, p. 191-201, 1940.

115. ZoBell, Claude E. Effect of oxygen tension on the rate of oxidation of organic matter in sea water by bacteria. *Jour. Marine Research*, v. 3, p. 211-223, 1940.

116. ZoBell, Claude E. The Scripps Institution of Oceanography. *Turtox News*, v. 19, p. 22-23, 1941.

117. Fox, Denis L. Changes in the tissue chloride of the California mussel in response to heterosmotic environments. *Biol. Bull.*, v. 80, p. 111-129, 1941.

118. Shepard, Francis P., and K. O. Emery. Submarine topography off the California coast: Canyons and tectonic interpretations. *Geo . Soc. Amer., Spec. Pap.*, no. 31, 171 pp., 1941. (*Not available for exchange.*)

119. Monk, Cecil R. Marine Harpacticoid copepods from California. *Amer. Microsc. Soc., Trans.*, v. 60, p. 75-99, 1941.

120. McEwen, George F. Observations on temperature, hydrogen-ion concentration, and periods of stagnation and overturning in lakes and reservoirs of San Diego County, California. *SCRIPPS INSTITUTION BULLETIN*, v. 4, no. 9, p. 219-260, 1941.

121. Allen, W. E. Classification and taxonomy. *Turtox News*, v. 19, p. 73-75, 1941.

Number

122. Allen, W. E. Ocean pasturage in California waters. *Scientific Monthly*, v. 52, p. 261-264, 1941.

123. Munk, W. H. Internal waves in the Gulf of California. *Jour. Marine Research*, v. 4, p. 81-91, 1941.

124. ZoBell, Claude E. Studies on marine bacteria. I. The cultural requirements of heterotrophic aerobes. *Jour. Marine Research*, v. 4, p. 42-75, 1941.

125. Gordon, Robert D. The estimation of a quotient when the denominator is normally distributed. *Annals of Math. Statistics*, v. 12, p. 115-118, 1941.

126. Shepard, F. P., K. O. Emery, and E. C. La Fond. Rip currents: A process of geological importance. *Jour. Geol.*, v. 49, p. 337-369, 1941.

127. Emery, K. O., and R. H. Tschudy. Transportation of rock by kelp. *Geol. Soc. Amer.*, Bull., v. 52, p. 855-862, 1941.

128. Allen, W. E. Frustules of marine plankton diatoms. *Chronica Botanica*, v. 6, p. 365-367, 1941.

129. Sverdrup, H. U. Water masses and currents of the North Pacific Ocean. *Science*, v. 93, p. 436, 1941.

130. Sverdrup, H. U. An analysis of the ocean currents off the American west coast between 40°N and 40°S. *Univ. Southern California, Chronicle Ser.*, p. 17-20, 1941.

131. Sargent, Marston C. The effect of light intensity on the configuration of the photosynthetic mechanism. *Chronica Botanica*, v. 6, p. 347, 1941.

132. Fox, Denis L., and Bradley T. Scheer. Comparative studies of the pigments of some Pacific Coast echinoderms. *Biol. Bull.*, v. 80, p. 441-455, 1941.

133. Sverdrup, H. U. The influence of bottom topography on ocean currents. p. 66-75 in *Contributions to Applied Mechanics and Related Subjects, Theodore von Kármán Anniversary Volume, Pasadena, California Inst. Technology*, 337 pp., 1941.

134. Fox, D. L., and C. F. A. Pantin. The colours of the plumose anemone *Metridium senile*. *Roy. Soc. London, Trans., Ser. B.*, v. 230, p. 415-45, 1941. *(Not available for exchange.)*

135. Sumner, F. B. Is evolution inscrutable? *Science*, v. 93, p. 521-522, 1941.

136. Sverdrup, H. U., and R. H. Fleming. The waters off the coast of southern California, March to July, 1937. *SCRIPPS INSTITUTION BULLETIN*, v. 4, no. 10, p. 261-378, 1941.

137. Fox, Denis L., and Lloyd J. Anderson. Pigments from marine muds. *Nat. Acad. Sci., Proc.*, v. 27, p. 333-336, 1941.

138. Emery, K. O. Rate of surface retreat of sea cliffs based on dated inscriptions. *Science*, v. 93, p. 617-618, 1941.

139. Emery, K. O. Transportation of rock particles by sea-mammals. *Jour. Sedim. Petrology*, v. 11, p. 92-93, 1941.

Number

140. Allen, W. E. Depth relationships of plankton diatoms in sea water. *Jour. Marine Research*, v. 4, p. 107-111, 1941.

141. Tibby, Richard B. The water masses off the west coast of North America. *Jour. Marine Research*, v. 4, p. 112-121, 1941.

142. Sverdrup, H. U., and Staff. Research within physical oceanography and submarine geology at the Scripps Institution of Oceanography during April 1940 to April 1941. *Amer. Geophys. Un., Trans., 1941*, p. 490-494, 1941.

143. ZoBell, Claude E. Kelp cutting and its consequences. *California Conservationist*, Sacramento, 3 pp., 1941.

144. Revelle, Roger. Criteria for recognition of sea water in ground-waters. *Amer. Geophys. Un., Trans.*, 1941, p. 593-597, 1941.

145. Coe, Wesley R. Sexual phases in wood-boring mollusks. *Biol. Bull.*, v. 81, p. 168-176, 1941.

146. Shepard, Francis P. Nondepositional physiographic environments off the California coast. *Geol. Soc. Amer. Bull.*, v. 52, p. 1869-1886, 1941.

147. Sverdrup, H. U. The Pacific Ocean. *Science*, v. 94, p. 287-293, 1941.

148. Emery, K. O., and R. S. Dietz. Gravity coring instrument and mechanics of sediment coring. *Geol. Soc. Amer., Bull.*, v. 52, p. 1685-1714, 1941.

149. Gordon, Robert D. Values of Mills' ratio of area to bounding ordinate and of the normal probability integral for large values of the argument. *Annals Math. Statistics*, v. 12, p. 364-366, 1941.

150. ZoBell, Claude E. Apparatus for collecting water samples from different depths for bacteriological analysis. *Jour. Marine Research*, v. 4, p. 173-188, 1941.

151. Allen, Winfred E. Twenty years' statistical studies of marine plankton dinoflagellates of southern California. *Amer. Midland Naturalist*, v. 26, p. 603-635, 1941.

152. ZoBell, Claude E., and Catharine B. Feltham. The bacterial flora of a marine mud flat as an ecological factor. *Ecology*, v. 23, p. 69-78, 1942.

153. Fox, Denis L., and J. F. Danielli. Surface chemistry of carotenoids. I. Astacene. *Biochem. Jour.* (London), v. 35, p. 1388-1395, Dec., 1941.

154. Fox, Denis L., and Sheldon C. Crane. Concerning the pigments of the two-spotted octopus and the opalescent squid. *Biol. Bull.*, v. 82, p. 284-291, 1942.

155. Shepard, Francis P., and Eugene C. La Fond. Mean sea level and sand movement: A reply. *Science*, v. 95, p. 193-194, 1942.

156. Coe, Wesley R. The reproductive organs of the prosobranch mollusk *Crepidula onyx* and their transformation during the change from male to female phase. *Jour. Morphology*, v. 70, p. 501-512, 1942.

157. Sumner, Francis B., and Urless N. Lanham. Studies of the respiratory metabolism of warm and cool spring fishes. *Biol. Bull.*, v. 82, p. 313-27, 1942.

Number

158. Jacobs, Woodrow C. On the energy exchange between sea and atmosphere. *Jour. Marine Research*, v. 5, p. 37-66, 1942.

159. ZoBell, Claude E. Microorganisms in marine air. *A.A.A.S. Publ.* no. 17, p. 55-68, 1942.

160. Dietz, R. S., K. O. Emery, and F. P. Shepard. Phosphorite deposits on the sea floor off southern California. *Geol. Soc. Amer., Bull.*, v. 53, p. 815-848, 1942.

161. Coe, Wesley R., and Denis L. Fox. Biology of the California sea-mussel (*Mytilus californianus*). I. Influence of temperature, food supply, sex, and age on the rate of growth. *Jour. Exper. Zool.*, v. 90, p. 1-30, 1942.

162. Coe, Wesley R. Influence of natural and experimental conditions in determining shape of shell and rate of growth in gastropods of the genus Crepidula. *Jour. Morphology*, v. 71, p. 35-51, 1942.

163. Johnson, Martin W. Some observations on the feeding habits of the octopus. *Science*, v. 95, p. 478-479, 1942.

164. Sverdrup, H. U. Oceanography for meteorologists. *New York, Prentice-Hall.* 246 pp., 1942. (*Not available for exchange.*)

165. Sverdrup, H. U., and Staff. Oceanographic observations on the *E. W. Scripps* cruises of 1938. *SCRIPPS INSTITUTION, RECORDS OF OBSERVATIONS*, v. 1, no. 1, 63 pp., 1942.

166. Johnson, Martin W. Concerning the hitherto unknown males of the copepods *Microsetella rosea* (Dana), *Vettoria granulosa* (Giesbrecht), and *Corissa parva* Farran. *Amer. Micros. Soc.*, Trans., v. 61, p. 430-437, 1942.

167. Fox, Denis L. The carotenoid pigments of cryptogams. *Chronica Botanica*, v. 7, p. 196-198, 1942.

168. Grant, Carroll W. Studies in marine microbiology at the Scripps Institution of Oceanography, University of California, La Jolla. *Amer. Phil. Soc.*, Year Book, 1941, p. 157-158, 1942.

169. ZoBell, Claude E. Bacteria of the marine world. *Scientific Monthly*, v. 55, p. 320-330, 1942.

170. Johnson, Martin W., and Weldon M. Lewis. Pelagic larval stages of the sand crabs *Emerita analoga* (Stimpson), *Blepharipoda occidentalis* Randall, and *Lepidopa myops* Stimpson. *Biol. Bull.*, v. 83, p. 67-87, 1942.

171. Gilbert, J. Y. The errors of the Sedgwick-Rafter counting chamber in the enumeration of phytoplankton. *Amer. Micros. Soc.*, Trans., v. 61, p. 217-226, 1942.

172. Sumner, Francis B. Where does adaptation come in? *Amer. Naturalist*, v. 76, p. 433-444, 1942.

173. ZoBell, Claude E., and Carroll W. Grant. Bacterial activity in dilute nutrient solutions. *Science*, v. 96, p. 189, 1942.

Number

174. Doudoroff, Peter. The resistance and acclimatization of marine fishes to temperature changes. I. Experiments with *Girella nigricans* (Ayres). *Biol. Bull.*, v. 83, p. 219-244, 1942.

175. Sverdrup, H. U., and Staff. Research within physical oceanography and submarine geology at the Scripps Institution of Oceanography during April 1941 to April 1942. *Amer. Geophys. Un.*, Transactions, p. 323-325, 1942.

176. Wohnus, J. Frederick. The kelp resources of southern California. *California Fish and Game*, v. 28, p. 199-205, 1942.

177. Wohnus, J. Frederick. The development of the sporophyte of *Macrocystis pyrifera*. *Turtox News*, v. 20, p. 133-136, 1942.

178. Sverdrup, H. U., M. W. Johnson, and R. H. Fleming. The oceans: Their physics, chemistry, and general biology. *New York, Prentice-Hall.* 1087 pp., 1942. (*Not available for exchange*).

179. ZoBell, Claude E., and Carroll W. Grant. The bacterial oxidation of rubber. *Science*, v. 96, p. 379-380, 1942.

180. Allen, W. E. Occurrences of "red water" near San Diego. *Science*, v. 96, p. 471, 1942.

181. ZoBell, Claude E. Changes produced by microorganisms in sediments after deposition. *Jour. Sedim. Petrol.*, v. 12, p. 127-136, 1942.

182. Grant, Carroll W., and Claude E. ZoBell. Oxidation of hydrocarbons by marine bacteria. *Soc. Exper. Biol. and Medicine, Proc.*, v. 51, p, 266-267, 1942.

183. Gilbert, J. Y., and W. E. Allen. The phytoplankton of the Gulf of California obtained by the *E. W. Scripps* in 1939 and 1940. *Jour. Marine Research*, v. 5, p. 89-110, 1943.

184. Fox, Denis L., and David M. Updegraff. Adenochrome, a glandular pigment in the branchial hearts of the octopus. *Archives of Biochemistry*, v. 1, p. 339-356, 1943.

185. Sverdrup, H. U., and Staff. Oceanographic observations of the Scripps Institution in 1939. *SCRIPPS INSTITUTION OF OCEANOGRAPHY, RECORDS OF OBSERVATIONS*, v. 1, no. 2, p. 65-159, 1943.

186. Shepard, Francis P. Shoreline erosion at La Jolla, California. *Amer. Soc. Civil Engineers, Jour.*, v. 13, p. 80-82, 1943.

187. Tschudy, Robert H., and Marston C. Sargent. Agar-bearing seaweeds at La Jolla. *Science*, v. 97, p. 89-90, 1943.

188. Allen, W. E. Microscopic creatures afloat in water. *Turtox News*, v. 21, p. 9-11, 1943.

189. Anderson, Lloyd, and J. C. Hindman. A versatile continuous reading thermionic voltmeter. *Industr. and Engin. Chem.*, Anal ed., v. 15, p. 42-45, 1943.

190. Cupp, Easter E. Marine plankton diatoms of the west coast of North America. *SCRIPPS INSTITUTION OF OCEANOGRAPHY, BULLETIN*, v. 5, p. 1-238, 1943.

191. Johnson, Martin W. Studies on the life history of the marine annelid *Nereis vexillosa*. *Biol. Bull.*, v. 84, p. 106-114, 1943.

Number

192. ZoBell, Claude E. Influence of bacterial activity on source sediment. *Oil Weekly*, 12 pp., April 26, 1943.

193. Coe, Wesley R. Development of the primary gonads and differentiation of sexuality in *Teredo navalis* and other Pelecypod mollusks. *Biol. Bull.*, v. 84, p. 178-186, 1943.

194. Sumner, F. B., and Peter Doudoroff. An improved method of assaying melanin in fishes. *Biol. Bull.*, v. 84, p. 187-194, 1943.

195. Sumner, F. B. A further report upon the effects of the visual environment on the melanin content of fishes. *Biol. Bull.*, v. 84, p. 195-205, 1943.

196. Novelli, G. David. Bacterial oxidation of hydrocarbons in marine sediments. *Soc. Exper. Biol. and Med., Proc.*, v. 52, p. 133-34, 1943.

197. Coe, W. R. Sexual differentiation in mollusks. I. Pelecypods. *Quart. Review of Biol.*, v. 18, p. 154-164, 1943.

198. Sargent, Marston C., and James C. Hindman. The ratio of carbon dioxide consumption to oxygen evolution in sea water in the light. *Jour. Marine Research*, v. 5, p. 131-135, 1943.

199. ZoBell, Claude E., and Carroll W. Grant. Bacterial utilization of low concentrations of organic matter. *Jour. Bacteriology*, v. 45, p. 555-564, 1943.

200. Graham, Herbert W. Chlorophyll-content of marine plankton. *Jour. Marine Research*, v. 5, p. 153-160, 1943.

201. Jacobs, Woodrow C. Sources of atmospheric heat and moisture over the North Pacific and North Atlantic Oceans. *New York Acad. Sci.*, Annals, v. 44, p. 19-40, 1943.

202. Sverdrup, H. U. On the ratio between heat conduction from the sea surface and heat used for evaporation. *New York Acad. Sci.*, Annals, v. 44, p. 81-88, 1943.

203. Young, R. T., Jr. A note on some laboratory measurements of the transparency of sea water. *Jour. Marine Research*, v. 5, p. 111-115, 1943.

204. ZoBell, Claude E. The effect of solid surfaces upon bacterial activity. *Jour. Bacteriology*, v. 46, p. 39-56, 1943.

205. Fox, Denis L., and Wesley R. Coe. Biology of the California sea-mussel (*Mytilus californianus*). II. Nutrition, metabolism, growth and calcium deposition. *Jour. Exper. Zoology*, v. 93, p. 205-249, 1943.

206. McEwen, George F. Statistical problems of standardizing the precipitation ratio and estimating its error. *Polish Inst. Arts and Sciences in America, Bull.*, v. 1, p. 1011-43, 1943.

207. Sverdrup, H. U., and Staff. Research within physical oceanography and submarine geology at the Scripps Institution of Oceanography during April 1942 to April 1943. *Amer. Geophys. Union.*, Trans., 1943, p. 244-46, 1943.

208. Worley, Leonard G. The structure and function of the Golgi system in the living cells of developing molluscs. *Nat. Acad. Sci.*, Proc., v. 29, p. 225-228, 1943.

209. Worley, Leonard G. The relation between the Golgi apparatus and "droplets" in the cell stainable vitally with methylene blue. *Nat. Acad. Sci.*, Proc., v. 29, p. 228-231, 1943.

Number

210. ZoBell, Claude E., Carroll W. Grant, and Herbert F. Haas. Marine micro-organisms which oxidize petroleum hydrocarbons. *Amer. Assoc. Petr. Geol.*, Bull., v. 27, p. 1175-1193, 1943.

211. Worley, Leonard G., and Elizabeth K. Worley. Studies of the supravitally stained Golgi apparatus. I. Its cycle in the tectibranch mollusc, *Navanax inermis* (Cooper). *Jour. Morphology*, v. 73, p. 365-398, 1943.

212. Graham, Herbert W. *Gymnodinium catenatum*, a new dinoflagellate from the Gulf of California. *Amer. Microscop. Soc.*, Trans., v. 72, p. 259-261, 1943.

213. Allen, W. E. "Red water" in La Jolla Bay in 1942. *Amer. Microscop. Soc.*, Trans., v. 72, p. 262-264, 1943.

214. Shepard, Francis P. Imaginary submarine canyons. *Science*, v. 98, p. 208-209, 1943.

JUN 3 1956

PAGE-WELD
BINDING
Patent Applied Fo